Iracilda Zeppone Carlos *Editor*

Sporotrichosis

New Developments and Future Prospects

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To my lovely husband Manoel and our sons,
Julio, Estevão, and Manoela

Preface

Until recently, sporotrichosis has been considered a neglected disease. Despite its worldwide spread with numerous hyperendemic areas, and being the most frequent subcutaneous mycosis in large geographical areas such as South America, as well as outbreaks occurring in several tropical and subtropical countries, references in the basic literature citing sporotrichosis as an example of human mycosis are still rare. Several factors have contributed to an increased interest in this disease. As an opportunistic infection, its incidence has been associated with risk groups such as patients infected with human immunodeficiency virus or receiving immunosuppressive therapies, and patients with chronic diseases, among others.

Another point of great interest regards sporotrichosis becoming an important zoonosis. This has changed the classic epidemiological pattern shaped by many years of soil-associated transmission, frequently through punctures from thorns; hence the name 'rose gardener's disease'.

Several advances have been made in terms of comprehending the biology of *Sporothrix schenckii* and the pathogenesis of sporotrichosis. This book aims to consolidate these major advances to date. Subsequently, nine chapters were conceived to cover the main areas in which the greatest progress has been made and highlighting others needing further development. In Chap. 1, a brief history of the disease is given, from its first description by Benjamin Schenck until the current state of its geographic distribution. Chapter 2 is devoted to the description of the causative agent and the progress made from its description until the recent identification of other species collectively known as the *S. schenckii* complex. Chapter 3 is dedicated to the structural aspects of the fungus's immunomodulatory components that determine the immune response against *S. schenckii*; also mentioned are some of the virulence factors, which are further expanded in the next chapter. Chapter 4 refers to the aspects of interaction between *S. schenckii* and its environmental niche and how this interaction can determine the host response. The clinical aspects of human and animal sporotrichosis are embraced in Chaps. 5 and 6, respectively, whereas Chap. 7 is devoted to the more relevant aspects of the immune response against *S. schenckii*, mostly from results reported from our

laboratory. Chapter 8 covers the immunological diagnosis of the disease, and the last chapter describes current therapies and new developments in the fields of antifungal and immunostimulatory treatments.

It is our goal that this work serves as a reference for the study of sporotrichosis by medical students, general practitioners, infectious disease specialists, microbiologists, biomedical researchers, and others interested in this area. As the first edition and as an area of intense current investigation, this may not be a perfect work. We are aware that future editions will have to improve in several aspects, but with this first effort we hope to at least contribute to the scientific community learning more about this emerging disease and hopefully stimulate the interest of new research groups in order to sprout new research projects that will help to seek new and more effective tools for the diagnosis, prevention, and treatment of sporotrichosis.

I thank all the authors who contributed directly with their experience in writing the various chapters, as well as all those whose work, featured or not, allowed the existence of this book and also those who gave their suggestions for generally improving the book. Thanks also go to our numerous master and PhD students, postdoctoral researchers, and other collaborators over the years whose projects gave rise to many of the findings presented here. Finally, I dedicate a special acknowledgement to my friend Marisa Campos Polesi for the more than 20 years dedicated to the Immunology Laboratory of the School of Pharmaceutical Sciences, UNESP, and also for our equally long and lasting friendship, both being important elements driving my scientific accomplishments throughout these years. To all of them, my many thanks.

Araraquara, SP, Brazil Iracilda Zeppone Carlos

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About the Editor

Iracilda Zeppone Carlos is Titular Professor of the Clinical Immunology Laboratory of the School of Pharmaceutical Sciences, São Paulo State University Júlio Mesquita Filho (UNESP), where her research is focused on the study of the cell-based immune response applied to the mechanisms and pathogenesis of experimental diseases, including cancer and fungal diseases. Her interest in the field of cellular immunity began with her studies on experimental sporotrichosis since 1986, linked to her doctoral thesis. She is heavily involved in a variety of research fields, including experimental sporotrichosis and the anti-inflammatory and anti-tumoral activities of natural products. Her research has been focused on the innate and adaptive immune responses against *Sporothrix schenckii* and, more recently, on the relationship between the environment and the immunopathogenesis of sporotrichosis. All these different approaches have opened new areas in her lab committed to the search of new prophylactic and therapeutic tools against this disease. She has contributed to the scientific formation of more than 60 researchers, including the supervision of nearly 50 MsC/PhD theses, mostly regarding the immunology of sporotrichosis.

Chapter 1
Sporotrichosis: An Emergent Disease

Iracilda Zeppone Carlos and Alexander Batista-Duharte

Abstract In recent decades, the frequency of invasive fungal infections has increased steadily, resulting in considerable morbidity and mortality. The increasing number of fungal infections has significantly contributed to health-related costs. The outcome of an infection with a human-pathogenic fungus often depends on the immune status of the host organism. Patients with a weakened immune system are at high risk of developing a serious fungal infection. On the other hand, increased prescribing of antifungals has led to the emergence of resistant fungi, resulting in treatment challenges. *Sporothrix* complex is an environmental pathogenic fungus found worldwide in soil, plants, and decaying vegetables. It is the etiological agent of sporotrichosis in humans and several domestic animals. Sporotrichosis was a neglected disease; however, it is now considered an emergent disease and is causing concern for the health authorities of several tropical and subtropical countries such as Brazil, Mexico, and Peru. Over the past decade, the incidence of sporotrichosis has been on the rise, and currently different fungal genotypes may be closely associated with the virulence of this fungus. Some leisure and occupational activities, such as agriculture and floriculture, have been associated with transmission of the disease, but today it is considered an important zoonosis, particularly in Brazil. This chapter also presents cases of sporotrichosis reported worldwide to show a picture of this disease.

Keywords Sporotrichosis • *Sporothrix schenckii* • Emergent disease • Outbreaks

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1.1 Introduction

Diseases are qualified as "emergent" if they have recently become a cause for concern because of an increase in virulence, infection of a novel host, and/or occurrence in a new area. According to the US Centers for Disease Control and Prevention, emergent diseases are new or known infectious diseases, the incidence of which has increased over the past 2 decades. The increase in fungal infections over the past few decades is striking. The emergence of new fungal pathogens and the resurgence of mycotic diseases that had previously been uncommon is a serious and growing public health problem. The re-emergence or emergence of opportunistic mycoses is facilitated by not only climatic change and human interventions related to medical therapy modifying the biotope but also the rapid spread of treatment-resistant strains and the increasing size of the population at risk suffering immunological disorders—individuals with AIDS, recipients of solid organ or hematopoietic stem cell transplants, patients with hematologic malignancies, and other individuals receiving immunosuppressive treatment.

Sporotrichosis (also known as rose gardener's disease) is a deep cutaneous mycotic infection caused by the dimorphic fungus *Sporothrix schenckii*. Over several decades, the fungus was mistakenly included in the genus *Sporotrichum*. This designation remained until 1962, when Carmichael reported differences between the genus *Sporotrichum* and the isolated instances of a patient with sporotrichosis (Carmichael 1962). The generic type species *S. schenckii* is characterized by tear-shaped conidia on small, clustered denticles (de Hoog et al. 2000). The fungus is thermally dimorphic; that is, at 37 °C, a yeast-like phase is produced under appropriate conditions (Howard 1961; de Hoog 1974; Travassos and Lloyd 1980). Molecular phylogenetic analyses have shown that several species exist within the *S. schenckii* species complex (de Beer et al. 2003; Marimon et al. 2006, 2007; Madrid et al. 2010; Criseo and Romeo 2010). Multilocus sequence data proved to be supported by small phenotypic characteristics that led to the description of the following novel clinically relevant species: *S. brasiliensis*, *S. globosa*, and *S. mexicana*, in addition to *S. schenckii sensu stricto* and *S. luriei* (Marimon et al. 2007, 2008). Several non-pathogenic environmental species have been recently described, including *S. stylites*, *S. lignivora*, *S. humicola* (de Meyer et al. 2008), *S. variecibatus* (Roets et al. 2008), *S. brunneoviolacea* and *S. dimorphospora* (Madrid et al. 2010). The species differ significantly in virulence, predilection (Marimon et al. 2007; Arrillaga-Moncrieff et al. 2009; Fernández-Silva et al. 2012; Fernandes et al. 2013), and geographic distribution (Rodrigues et al. 2013a, b).

Sporotrichosis was first recognized in December 1896 by Benjamin Robinson Schenck, a medical student, while he was working with Simon Flexner in the Johns Hopkins University and Hospital in Baltimore, Maryland, USA. He isolated the fungi from ulcerative nodules in the fingers and arms of a 36-year-old patient. The sample was sent to the mycologist Erwin Smith to be identified, and he concluded that the fungus belonged to the genus *Sporotrichum.* This report was published in 1898, the year in which Schenck graduated in medicine (Schenck 1898). The second reported case of sporotrichosis was in 1900 in a child who suffered a lesion in the hand from

a hammer. The lesion spontaneously regressed, and in this case the fungus was classified with the current designation *S. schenckii* (Hektoen and Perkins 1900).

Sporotrichosis has traditionally been associated with agricultural work, since the causative agent is found naturally in soil. However, cases have been reported recently in urban areas related to zoonotic transmission. Sporotrichosis exhibits several clinical forms and it is well known that the clinical form of sporotrichosis depends on the host's immune condition (Kong et al. 2006). Cutaneous sporotrichosis is the most common form, including fixed cutaneous sporotrichosis (FCS) and lymphocutaneous sporotrichosis (LS), whereas disseminated sporotrichosis (DS) is infrequent (Morgado et al. 2011). DS is reported mostly among immunocompromised hosts. An association between AIDS and DS has been reported since the discovery of AIDS in the early 1980s (Matter et al. 1984; Romero-Cabello et al. 2011). The emergence of DS in immunocompromised patients has highlighted the need for more effective treatments. In addition to immunological competence, different fungal genotypes may be closely associated with the virulence of different clinical forms of *S. schenckii* infection (Kong et al. 2006). Sporotrichosis has been reported in cats, dogs, horses, cows, camels, dolphins, goats, mules, birds, pigs, rats, and armadillos, as well as in humans. Sporotrichosis is currently an important zoonosis, with animal-to-human transmission well documented (Silva et al. 2012).

Increases in isolated cases and local sporotrichosis outbreaks in different geographic areas have prompted research on the biology of *S. schenckii* species complex, host immune response, and diagnostic, preventive, and therapeutic tools. Interest in the emergence of sporotrichosis is evidenced by the increasing number of scientific publications by year in this area (Fig. 1.1).

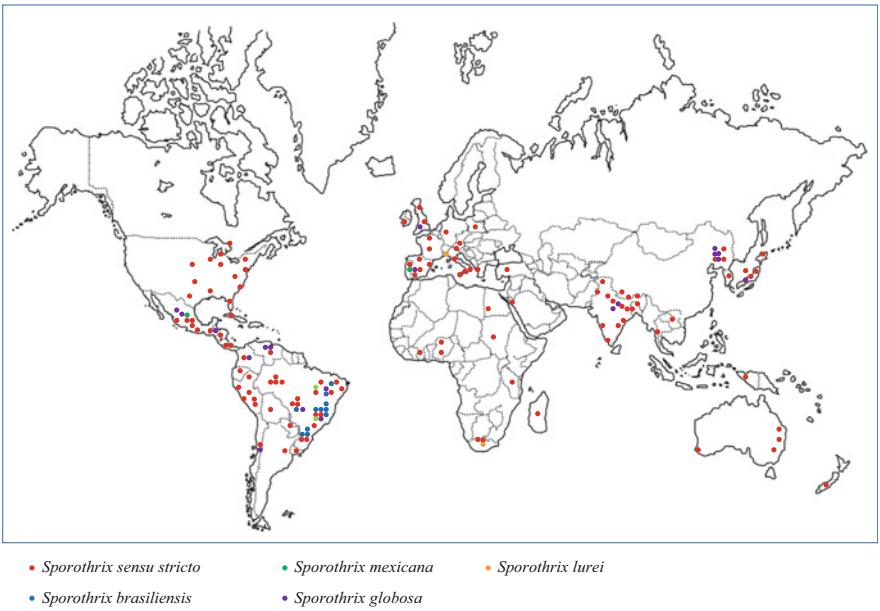

Fig. 1.1 Historical frequency of papers in sporotrichosis by years (Source: Pubmed)

1.2 Geographic Areas

S. schenckii species can be found worldwide (Fig. 1.2). However, despite sporotrichosis being globally distributed, its incidence differs and is a public health problem in well-defined geographic areas, mainly in tropical and temperate zones characterized by high humidity (80–95 %) and mild temperatures (25–28 °C). Therefore, the disease is regarded as hyperendemic in certain tropical and subtropical areas, particularly South America (Mackinnon 1972; Conti Díaz 1989; Mata-Essayag et al. 2013; McCarty and Pappas 2014).

A study revealed differences in geographical distribution among members of *S. schenckii* complex, including widespread and geographically restricted species. *S. brasiliensis* and *S. mexicana* occurred only in Brazil and Mexico, respectively. Interestingly, in contrast to *S. schenckii s. str.* and *S. brasiliensis*, which have been associated with both localized and invasive disease, no cases of invasive infections have been attributed to *S. globosa* (Marimon et al. 2007). An obstacle preventing comprehensive epidemiological investigation of sporotrichosis in developing countries is that the disease is probably greatly under-diagnosed. Moreover, the disease is not reportable in most countries. Thus, reliable data on incidence are not available from any region of endemicity (Pappas et al. 2000). Several studies provide evidence of the strong regional specificity of molecular types of *S. schenckii* (Mesa-Arango et al. 2002; Mora-Cabrera et al. 2001; Arenas et al. 2007; Rodrigues et al. 2014).

Several factors may explain the endemicity of sporotrichosis in developing countries: adults and children spend many hours in outdoor activities and are frequently exposed to punctures from thorns, splinters, and cuts from sedge barbs, handling of reed, sphagnum moss, hay, grasses (Bhutia et al. 2011; Dias et al. 2011) and by direct contact with domesticated infected animals such as dogs and cats (Nusbaum et al. 1983; Pereira et al. 2014). The most affected animal species is the cat, which can also exhibit a wide spectrum of clinical signs, ranging from a single cutaneous lesion to a disseminated form (Schubach et al. 2004; Pereira et al. 2011).

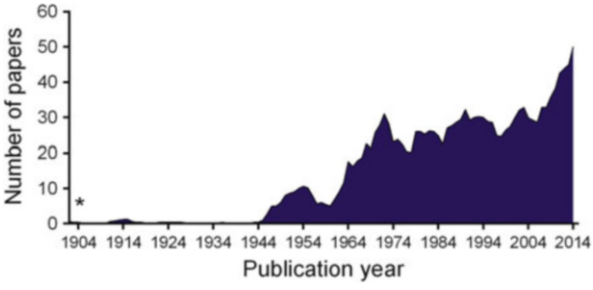

Fig. 1.2 World distribution of reported cases of sporotrichosis

1.2.1 USA and Canada

According to scientific literature, the first cases of sporotrichosis were those reported in the USA by Schenck in 1898 and 2 years later by Hektoen and Perkins (1900). One century later, between spring and summer 1988, several cases of sporotrichosis began to appear among forestry workers participating in annual reforestation programs in New York and Illinois. This was the largest recorded epidemic of sporotrichosis in the USA (Coles et al. 1992). It involved 84 cases in 15 states (Dixon et al. 1991) and was related to contaminated sphagnum moss obtained from a single Wisconsin distributor.

In addition to these reported human cases, several isolated outbreaks have been reported in several other states, including Mississippi, Oklahoma, Indiana, Texas, Virginia, Kansas, Vermont, Delaware, Alabama, and Florida (D'Alessio et al. 1965; Dahl et al. 1971; Park et al. 1972; Powell et al. 1978; Dooley et al. 1997; Laur et al. 1979; Dixon et al. 1991). However, despite these, no significant epidemiological studies have been carried out on a larger scale in the USA (McCarty and Pappas 2014).

The first case of sporotrichosis in Canada was reported by George E. Learmonth in 1911 (Learmonth 1915). He described as the causal agent a fungus bearing some resemblance to *S. beurmanni*, which was thought at the time to differ from *S. schenckii* (Rippon 1988). After four decades, Hawks (1955) reported three cases of sporotrichosis and showed that infection with the fungus *S. schenckii* is not too uncommon, depending on the analyzed area. Carr et al. reviewed a series of seven cases from various Toronto teaching hospitals and compared them with the world literature (Carr et al. 1995). In another study, DS was described in a patient with underlying hairy cell leukemia, with significant burden of infection evidenced by the isolation of *S. schenckii* from cutaneous lesions, sputum, and blood (Bunce et al. 2012).

1.2.2 Latin America

Sporotrichosis is essentially endemic in Latin America. Several countries, including Brazil, Mexico, and Peru, have an elevated incidence and prevalence of the disease, whereas others exhibit different numbers of annual cases. Nowadays, cases have been reported in almost all Latin American countries.

In Mexico, sporotrichosis was first reported by Gayon in 1913. Mexico is now one of the countries with a higher incidence of the disease. The major endemic area is the western part of the country (State of Jalisco) (Bonifaz et al. 2007). Mayorga Rodríguez et al. reported the largest series of cases in this country. A total of 822 cases of sporotrichosis with positive cultures were analyzed in a 37-year review of mycological files in various public and private institutions in the city of Guadalajara, Jalisco. The most frequent clinical form was LS (localized in the upper

extremities), which affected both sexes almost equally, with age ranging from 1 to 15 years. In terms of occupation, housewives showed the highest incidence. The cases reported were from five states; 539 cases were filed in 54 municipalities of Jalisco state (Mayorga-Rodríguez et al. 1997). Another study revealed that 1 in 25 isolated instances from Mexico (4 %), one in three isolated instances from Guatemala (33.3 %), and two in four isolated instances from Colombia (50 %) belonged to *S. globosa*, whereas the other isolated instances belonged to *S. schenckii sensu stricto*. This was the first record of *S. globosa* in Mexico and Central and South America (Magand et al. 2009).

There are several reports of sporotrichosis in Central America. The first cases were diagnosed in the isthmus of Panama. The clinical diagnosis was confirmed in each case by the isolation of *S. schenckii* from pus aspirated from the undrained nodule and cultured at a room temperature of 37 °C. Skin tests with sporotrichin were positive (Calero and Tapia 1962). Mayorga et al. (1979) described an endemic area of sporotrichosis in Guatemala, where a high percentage of patients, from whom *S. schenckii* was isolated, were injured when handling fish.

A 35-year-old male born in Nicaragua but residing in Spain suffered from nodular lesions on the right forearm after trauma with laceration in the right hand while working with a shear in a field in his native country 4 years previously. His lesions were treated locally, but in the following months an ascendant nodular lesion appeared along the arm, some ulcerated, with periods of improvement and worsening. He received several courses of antibiotics without any successful response. He never had fever or other systemic symptoms. The Sabouraud agar culture showed at 30 °C brownish filamentous structures, showing the presence of the fungus *S. schenckii* (Pérez-Fernández et al. 2013).

In Venezuela, sporotrichosis was first described in 1935; it is now the second most common subcutaneous mycosis after chromomycosis and is regarded as endemic. To date there have been no reports of disseminated forms of the disease, even amongst patients with AIDS. Mata-Essayag et al. (2013) reviewed the literature and found that 133 sporotrichosis cases had been diagnosed in Venezuela. Most patients were aged <30 years (66.15 %); transmission was not identified in 61.6 % of these patients. The predominant clinical form in this population was LS (63.15 %). Distribution by gender in Venezuela shows a predilection for male (71.4 %) compared with female (28.6 %) patients. This corresponds with the published literature, with the exception of studies by Espinosa-Texis et al. (2001), Kusuhara et al. (1988), and Mahajan et al. (2005). The apparent differences in gender distribution of the disease are probably related to occupation and exposure. The authors observed that sporotrichosis can occur in any age group, with most patients aged <30 years (65.9 %), 34.58 % of whom were aged <15 years. These data are very similar to other reports in the literature (Pappas et al. 2000; Ramírez et al. 2011). A recent study of molecular epidemiology of human sporotrichosis in Venezuela revealed that sporotrichosis in Venezuela is caused at least by two species: *S. schenckii s. str.* and *S. globosa*, the latter representing the highest incidence reported in the Americas (30 %) (Camacho et al. 2015).

The first case of sporotrichosis in Peru was described by Edmundo Escomel in 1909 (reviewed in Sánchez-Saldaña et al. 2009). It is now an important mycosis in this country. In 2000, Pappas reported epidemiological, clinical, and treatment data from 238 cases of culture-proven sporotrichosis from a relatively remote area of the south central highlands of Peru named Abancay, retrospectively collected during 1995–1997. Most cases (60 %) occurred in children aged <14 years, and the most commonly affected anatomic site was the face. Extracutaneous disease was rare in this population (Pappas et al. 2000).

Another study in South America found the incidence of LS in the area of Abancay, Peru, to be even higher than previously estimated (Pappas et al. 2000) and, unlike the situation in other parts of the world, children were disproportionately affected (Kusuhara et al. 1988; Vismer and Hull 1997; Ghosh et al. 1999). Many of the risk factors identified in this study, whether environmental, occupational, or socioeconomic, may be modifiable, which has important implications for the design of prevention strategies in this area of hyperendemicity. Population-based surveillance and a case–control study were conducted in Abancay to estimate the burden of disease and to determine risk factors for sporadic LS. Identified risk factors included owning a cat, playing in crop fields, having a dirt floor in the house, working mainly outdoors, and having a ceiling made of raw wood or conditions associated with a lower socioeconomic status. Decreased environmental exposure, such as wearing protective clothing during construction activities for adults, or limiting contact with cats and soil for children, and improvements in living spaces may decrease the incidence of LS (Lyon et al. 2003).

The automated Amplified Fragment Length Polymorphism (AFLP) analysis is a valuable tool for further investigation of the epidemiology and ecology of *S. schenckii*. It was used to analyze the genetic diversity of Peruvian strains of *S. schenckii* and to compare them to a panel of non-Peruvian strains. The two populations identified in Peru occupied two distinct niches in the environment of patients (Neyra et al. 2005). Investigations of the fungi *S. schenckii* in relation to its interaction with the host and environment have provided valuable information for the prevention and control of this disease. However, a major obstacle is that incidence data for sporotrichosis in Peru are unreliable because diagnostic coverage is limited and reporting of the disease is not obligatory. In the Abancay province, major sporotrichosis investigations have been possible because of the existence of a referral center, providing excellent opportunities for clinical and epidemiological observations regarding the disease (Zurita 2014). Other regions in Peru in which cases have been detected are Lima, Loreto, Apurimac, Puno, Ayacucho, Amazonas, Cajamarca, Cusco, and San Martín (Neyra et al. 2005).

In Brazil, isolated cases, small outbreaks, and case series have frequently been reported. In 1907, Lutz and Splendore reported a first Brazilian case of sporotrichosis and described the parasite form of the fungus. They also reported the connection between *Sporothrix* and animals (Lutz and Splendore 1907). The first occurrence of feline sporotrichosis naturally acquired in Brazil was reported by Freitas et al. (1956); it consisted of a series of eight cases in cats reported by the same authors, corresponding to the largest number to that time (Freitas et al. 1965).

In 1986, Dunstan et al. (1986) published the largest series involving humans and cats until 1980.

Since 1998, researchers from the Fiocruz Institute have been observing an increasing number of cases of sporotrichosis in the population of Rio de Janeiro, Brazil. Regarding this, Barros et al. (2001, 2008) suggested that feline transmission of sporotrichosis was associated with a large and long-lasting outbreak of the disease in Rio de Janeiro city and surroundings.

Silva et al. (2012) studied sporotrichosis in an urban area with an exploratory analysis of its socio-spatial distribution in Rio de Janeiro, Brazil, from 1997 to 2007, identifying the areas with the heaviest transmission. During the study period, 1848 cases of sporotrichosis were reported, predominantly in adult women not currently in the labor market. The leading source of infection was wounds caused by domestic cats, which contributed to the spread of sporotrichosis in this urban area (Bustamante and Campos 2001; Fleury et al. 2001; Souza et al. 2006). Freitas et al (2012) reported 21 cases of sporotrichosis in HIV patients in Rio de Janeiro, demonstrating a positive correlation between disseminated disease and immunosuppression in HIV patients.

The south-eastern part of Brazil has also been experiencing a very large epidemic due to zoonotic transmission (Schubach et al. 2004), with cats as the main vector through which the disease is transmitted to humans and other animals (Barros et al. 2004; Rodrigues et al. 2013a, b). One study describes the epidemiological, mycological, and histopathological characteristics of sporotrichosis in small animals over a 10-year observation period in the south of Brazil (2000–2010). It showed that 92 cats and 11 dogs from eight municipalities in Rio Grande do Sul state developed the disseminated cutaneous and fixed cutaneous forms of the disease in particular. Respiratory signs such as sneezing, serious nasal discharge, and dyspnea were found in about 57 % of the animals. The detection of *S. schenckii* in different clinical samples showed the highest isolation in the testicles (46.6 %), oral cavity (45.2 %), and conjunctival mucosa (38.1 %), and it emphasized the importance of laboratory tests for mycosis confirmation, particularly in dogs that develop clinical manifestations without the presence of cutaneous lesions (Madrid et al. 2012).

Due to the ongoing zoonotic epidemic that started in 1998, sporotrichosis has become very common in Brazil. This encompassed the first 4 years of the ongoing epidemic, noting a wide age range from children to the elderly with a predominance of women, particularly women aged >40 years engaged in domestic work, with the majority having experienced a cat-related trauma. The same predominance of women, mainly in those aged 40–59 years, has been seen, with domestic work being the most common profession. Investigation of cats associated with patients showed around half of them as being infected, with a range of one to 14 infected cats per dwelling; however, the average was just one infected cat. A 2002 report detailed *Sporothrix* isolation from both infected (148) and healthy (84) cats in Rio de Janeiro (Schubach et al. 2002). This study showed that all infected cats were culture positive for skin lesions, while the fungus was also isolated from nasal cavities (66 %), oral cavities (42 %), and nails (40 %). Only three of the healthy

cats, all of which were domiciliary contacts of the infected cats, had positive cultures, all from oral swabs. An investigation in São Paulo city cultured the claws of 120 primarily indoor cats over 12 months, with only one cat culturing positive (Borges et al. 2013).

In Brazil, all species of the pathogenic cluster have been reported. Oliveira et al. 2011 characterized *Sporothrix* strains isolated during the course of the Rio de Janeiro sporotrichosis epidemic. They observed that 206 (83.4 %) of the isolated instances were characterized as *S. brasiliensis*, 15 (6.0 %) as *S. schenckii*, and one (0.5 %) as *S. mexicana*. A total of 25 (10.1 %) isolated instances could not be identified according to their phenotype and were classified as *Sporothrix* spp. Results showed that *S. brasiliensis* is highly prevalent (96.9 %) among cats with sporotrichosis, whereas *S. schenckii* was identified only once. The genotype of *Sporothrix* from cats was found to be identical to *S. brasiliensis* from human sources, confirming that the disease is transmitted by cats. *S. brasiliensis* showed low genetic diversity compared with its sibling taxon *S. schenckii*. No evidence of recombination in *S. brasiliensis* was found by split decomposition or PHI-test analysis, suggesting that *S. brasiliensis* is a clonal species. Strains recovered in São Paulo (SP), Minas Gerais (MG), and Paraná (PR) states share the genotype of the Rio de Janeiro (RJ) outbreak, different from the Rio Grande do Sul (RS) clone. The occurrence of separate genotypes among strains indicated that the Brazilian *S. brasiliensis* epidemic has at least two distinct sources. It has been suggested that cats represent a major host and the main source of cat and human *S. brasiliensis* infections in Brazil (Rodrigues et al. 2013b).

In Argentina, Vidal and Rodríguez 1993 reported the existence of an endemic focus of sporotrichosis in the northwestern province of Santa Fe, Argentina, among hunters of armadillos (*Dasypus novemcinctus*). This was the second world record for micro endemics, similar to those occurring in Uruguay. Negroni et al (2007) reported the case of a 59-year-old female who consulted the Mycology Unit of the Infectious Diseases Hospital Francisco Javier Muñiz, filing a straight 3-month history of periocular dermatitis. This injury occurred approximately 2 weeks after an accident in which part of her face hit against an adobe wall, producing superficial wounds. As the skin lesion progressively increased in size, the woman went to a dermatology service, where a biopsy and histopathological study was performed in which an intense inflammatory reaction with epithelioid granuloma, giant cells, and the presence of neutrophils was observed. Periodic acid–Schiff (PAS) staining showed some PAS-positive, oval elements, 2×4 μm in diameter in the giant cells. A mycelial fungus was isolated in which macro and micro characters corresponded morphologically to *S. schenckii*. Later, the diagnosis was confirmed by obtaining a yeast form of this fungus. A skin test for sporotrichin showed a strong positive reaction (20 mm). The test is of no diagnostic value in highly endemic areas, but Buenos Aires is an endemic area with a low incidence of clinical cases in which a very positive response to the sporotrichin test and the presence of characteristic lesions were compatible with the suspected diagnosis (Negroni et al. 2007).

In Uruguay, abundant rains may enable a stronger proliferation of *Sporothrix* on its natural substrates, with a correspondingly greater risk of infection during armadillo hunting. This explains why wet summers are followed by a higher number of sporotrichosis cases than are dry summers. It is easily explained by the intensive hunting of armadillos around the Easter holiday and may contribute to variations in annual incidence (Conti Díaz 1989). The high frequency of asteroid bodies in the direct fresh examination of pus in human cutaneous sporotrichosis was studied in Uruguay, and consequently the value of this method in the systematic diagnosis of the disease was confirmed. Direct examination of fresh *S. schenckii* asteroid bodies were found in 85.7 % of cases, which enabled diagnosis of the disease. Cultures confirmed the diagnosis in 95.2 % of the patients (Civila et al. 2004).

In Chile, some sporadic cases of sporotrichosis have been reported (Flores et al. 1982; Escaffi et al. 2010). Recently reported was a case of sporotrichosis induced by *S. globo*sa related to a lymphocutaneous form of the disease in a 75-year-old patient who performed horticulture work. In March and July 2011, soil and plant debris from five sectors where the patient had worked were extracted. The soil samples were diluted and inoculated in Sabouraud agar with cycloheximide and chloramphenicol at 26 °C. The plant debris was directly inoculated in the same medium. Colonies suggestive of *Sporothrix* complex were reseeded in potato dextrose agar (PDA) at 26 °C and identified as recommended by Marimon et al. 2007. One colony of the ten plates from the first sampling was identified as *S. globosa*. For the first time in Chile a species of *Sporothrix* complex was isolated from the environment, as *S. globosa* was the species identified both in the ground and from the patient with sporotrichosis (Cruz et al. 2012).

Understanding of the role of healthy cats as potential disseminators of pathogens and opportunistic filamentous fungi to humans was obtained by Betancourt et al. in Temuco, Chile (Betancourt et al. 2013). A total of 50 tissue and hair samples were collected from dermatologically healthy cats and cultured in Sabouraud agar supplemented with glucose and chloramphenicol gentamicin (ASG), dermatophyte test medium (DTM) agar, and dermatophyte identification medium (DIM) and incubated at 28 °C for 21 days. The genus *Sporothrix* was demonstrated in the hair coat of dermatologically healthy cats, confirming their status as reservoirs and sources of opportunistic infection.

Other Latin American countries that have reported sporotrichosis cases are Guatemala, El Salvador, Costa Rica, Cuba, and Bolivia (Pardo-Castello and Trespalacios 1959; Azogue 1981; de Lezcano et al. 2008; Madrid et al. 2009; Pérez-Morales et al. 2014).

1.2.3 Europe

For reasons that are as yet unknown, sporotrichosis had dramatically reduced throughout Europe by the end of the Great War (1914–1918) (Alberici

et al. 1989) and any cases are now mainly observed in countries along the Mediterranean Sea (McCarty and Pappas 2014).

In France, Charles Lucien de Beurmann performed extensive pioneer research involving cutaneous aspects of sporotrichosis, and, with Henri Gougerot, published the monograph, *Les Sporotrichoses*, a treatise based on 250 cases of sporotrichosis in France (de Beurmann and Gougerot 1912). In 1903, dermatologist Raymond Sabouraud (1864–1938) suggested to Beurmann the use of potassium iodide for therapy. In 1906, Matruchot and Ramond, due to Beurmann's thorough research of sporotrichosis, suggested the term *Sporothrix beurmanni* be applied to the causal agent of the disease (Learmonth 1915). For this reason, *S. schenckii* was sometimes referred to as *Sporotrichum beurmanni*, causing confusion at that time.

A case of autochthonous French sporotrichosis in its fixed cutaneous form was reported in an immunocompetent patient. A 35-year-old French man presented with a cutaneous nodule on the right forefinger that had been there for 6 months. The patient's work involved contact with soil. He was diagnosed with FCS following isolation of *S. schenckii* in a skin biopsy culture in Sabouraud medium. The culture was negative for atypical mycobacteriosis. The patient recovered with oral itraconazole treatment. This diagnosis should be considered even if the patient has never been abroad to endemic areas, especially in the event of frequent exposure to soil and plants (Magand et al. 2009).

In Sanitária Virgen, Macarena, province of Seville, Spain, eight cases of cutaneous sporotrichosis confirmed via microbiological culture were reported from June 2006 to January 2010. All patients were healthy adult males, with an average age of 53.7 years. Overall, the lesions were located in the extremities: seven on upper limbs and one on a bottom limb, with the most frequent site being the forearm. Three of the four cases with a clear history of traumatic accident were professional or amateur gardeners in whom the trauma occurred with a rose; the fourth case was a farmer who had received scratches from orange thorns. Three of the remaining four lived in rural areas but did not remember any specific trauma. In the case of a 19-year-old patient, it was not possible to clarify the source of the infection. The most frequent clinical presentation in our patients was lymphocutaneous; exceptions were the patient with clinical presentation in the lower limbs and the patient from 2006, who had FCS (Ojeda et al. 2011).

Barile et al. (1993) reported 16 cases of cutaneous sporotrichosis observed in the province of Bari, southern Italy, in the period 1978–1992. No more than 55 cases have been documented in other European countries in the last 30 years; however, 58 cases (present series included) have been recorded in Italy over the same time period. Furthermore, 42 of them (73.7 %) originated in Apulia. This unexpectedly high incidence rate in Italy, and in Apulia in particular, provides evidence of the important role played by this area in the eco-epidemiology of sporotrichosis in Europe. In dogs, the first report of sporotrichosis in Apulia was a case of lymphocutaneous and nasal sporotrichosis in a hunting dog with a 3-month history of non-healing skin lesions. Cytological examination of nasal discharge and material collected from ulcerated skin surfaces showed a few cigar-shaped organisms within macrophages. Fungal cultures of nasal and ulcerated skin swabs yielded

colonies of *S. schenckii*. The dog received oral itraconazole for 7 days after therapy was instituted but died of unrelated causes. Necropsy examination was not performed (Cafarchia et al. 2007). No data on canine sporotrichosis have been reported so far in Apulia, a region where *S. schenckii* is considered to be endemic (Barile et al. 1993).

After the first case of sporotrichosis caused by *S. luriei* in South Africa, the second reported case was described in a patient living in Piacenza, Italy. Stained tissue sections (hematoxylin and eosin, and Gomori methenamine silver) revealed large hyaline thick-walled tissue form cells that had divided by septation or a budding process. The use of a fluorescent antibody reagent specific for *S. schenckii* confirmed the identity of the etiologic agent (Alberici et al. 1989). Other regions in Italy with reported cases are Pisa (Gori et al. 1997), Calabria, and Sicily (Criseo et al. 2008).

A 34-year-old man sought care at a podiatry clinic in Vila Nova de Famalicão, Portugal, in 2009 for multiple polymorphous eruptions and ulcers on both feet. No obvious cause of the disease could be observed. Although the patient had traveled to Malaysia in 2003 and had worn open footwear every day, he did not recall having any skin wound. In 2004, in Portugal, subcutaneous nodules appeared in both feet, became ulcerated, and spontaneously healed. By 2005, more severe lesions had appeared and became a chronic infection in both feet and lower limbs. The symptoms were diagnosed erroneously as dyshidrotic eczema, and treatment with topical corticosteroids was unsuccessful. The fungus had hyaline septated hyphae, with hyaline and dematiaceous conidia compatible with *Sporothrix* spp. Although this species has an atypical morphologic profile, a presumptive identification based on phenotypic characteristics allowed us to classify this fungus as *S. mexicana*. The diameter of colonies grown at 30 °C and 37 °C is smaller than those proposed by Marimon et al. but much closer to those of *S. schenckii* (Marimon et al. 2007). These differences could be attributable to the intraspecific variation of this single isolated instance.

Oliveira et al. (2014) characterized the first autochthonous case of human sporotrichosis reported in Lisbon, Portugal. Phenotypic and genotypic characterization revealed that the infection was caused by *S. globosa*. This report indicates that sporotrichosis may be under-diagnosed, particularly in Southern Europe and suggests Portugal as an emerging area for this fungal infection.

No cases of sporotrichosis in the British Isles were published until 1911. In that year, three cases were independently identified and reported by Walker in Edinburgh, von Ofenheim in Lewisham, and Adamson in London, whereas the first published case of sporotrichosis in Ireland was reported in 1918 by Wallace Beatty. The second Irish case was reported by Adamson 4 years later. In 1968, Symmers summarized the cases reported in this country until that year in (Symmers 1968). So far, no more cases have been reported in the Irish literature.

Other European countries with rare reported cases are Poland (Wroblewska et al. 2005), Austria (Staib et al. 1974), Czech Republic (Jirásek et al. 1976), and Germany (Krempl-Lamprecht 1978).

1.2.4 Africa

With the exception of a report from South Africa, there are very few reports of sporotrichosis in the African continent; thus, the distribution and pathogenicity of *S. schenckii* in this continent is not well known. Sporotrichosis in South Africa dates back to 1914 when the disease was first diagnosed in the gold mines. Occupational and recreational circumstances of infection are well established, and the environmental requirements for contracting the disease are better understood. Many outbreaks of sporotrichosis have been described in the literature (Hajjeh et al. 1997) and account for a significant proportion of reported cases. The largest reported outbreak occurred from 1941 to 1944 in South Africa; almost 3000 miners were infected, and the outbreak was attributed to contaminated mine timbers (Brown et al. 1947). Other important data from the African continent include the first case of sporotrichosis caused by *S. luriei* in a native of the Republic of South Africa who suffered a fixed type of sporotrichosis, as described by Ajello and Kaplan in 1969 (Ajello and Kaplan 1969).

Sporotrichosis cases were recorded in 42 suburbs in the greater Pretoria area as well as in 23 towns outside the Pretoria municipal boundary. It occurred in 154 patients, predominantly male, with ages ranging from less than 1 year to 90 years. Females in the area seemed to be at lesser risk, mainly becoming infected through gardening injuries, insect bites, or minor injuries due to outdoor activities. Exposure to possible sources of the fungus, either from recreational or occupational activities in males, was the main determining factor in acquiring the disease. The lymphocutaneous and localized forms of the disease were most often recorded (Vismer and Hull 1997).

Other African countries with some reports of sporotrichosis are Nigeria (Jacyk et al. 1981; Dalis et al 2014), Sudan (Gumaa 1978), Egypt (El-Mofty and Nada 1965), Congo (Callens et al. 2006), Tanzania (Pönnighaus et al. 2003), Ghana (Addy 1992), and Madagascar (Carod et al 2007).

1.2.5 Middle East

Sporotrichosis is rarely reported in Turkey. Only a few cases have been reported in the Turkish literature. Although sporotrichosis is rarely reported in our country, it should be considered in differential diagnosis in people working with soil and plants in cases with skin lesions after trauma and with chronic lymphocutaneous soft tissue infections (Gurcan et al. 2007). In Iran, Kazemi and Razi (2007) reported on a 23-year-old male florist gardener diagnosed with subcutaneous sporotrichosis caused by *S. schenckii*. The patient had a history of skin trauma caused by rose bushes when he was admitted to the medical mycology section of the School of Medicine. Despite worldwide distribution of sporotrichosis and other subcutaneous

mycoses, this type of infection has rarely been diagnosed in Iran: only seven human cases and one animal case of sporotrichosis have been previously reported.

1.2.6 Asia

Cases of sporotrichosis have been reported since 1964 in Asia (Oda et al. 1964). In 1996, Nakamura et al. identified the first case of feline sporotrichosis in Japan. Fortunately, no transmission to humans occurred in this case; however, the risk of humans contracting *S. schenckii* infection increases alongside increases in the number of animals with sporotrichosis. Inokuma et al. 2010 reported two cases of cutaneous sporotrichosis in Hokkaido: the first was a 55-year-old woman who presented in 2009 with a 6-month history of two dark red, crusted, infiltrated skin lesions measuring about 10 mm and 2 mm, respectively. The second patient was a 55-year-old man who presented with a chronic erosive nodule measuring 20×10 mm on the left mandible, which had been present for over 1 year in 2002. The authors suggest that the prevalence of cutaneous sporotrichosis in Hokkaido may be increasing as a result of recent global warming. On the other hand, an atypical case of sporotrichosis was presented by an elderly woman working as a horticulturist who had multiple ulcers and nodules on the face and the right upper back (Fujii et al. 2008).

A total of 165 sporotrichosis cases occurred in Nagasaki prefecture and were examined at Nagasaki University Hospital, Japan. Lesions were frequently seen on the face (49 cases, 29.5 %) and upper limbs (101 cases, 60.9 %). The localized cutaneous type of sporotrichosis (105 cases, 62.9 %) was much more frequent than LS (62 cases, 37.1 %) (Takenaka et al. 2014).

In some parts of China, especially northeast provinces—including Jilin, Heilongjiang, and Liaoning— sporotrichosis is regarded as endemic. Jilin is the most important agricultural province in northeast China, with about 187.4 thousand square kilometers in area with a population of 27 million. The climate is cold and dry in winter, yet hot and humid in summer. The average temperature is 6 °C for the whole year and −15 °C during the winter (Song et al. 2013). Several researchers also confirmed the presence of *S. schenckii* in the samples collected from different regions of Jilin (Zhang et al. 1996). Recently, the number of sporotrichosis cases has increased rapidly (Zhang et al. 2011; Gremião et al. 2015). The First Hospital of Jilin University, which is the central and biggest hospital in Jilin province, presented 457 sporotrichosis cases diagnosed by fungal culture from 2007 to 2009. In all, 89 (41.36 %) patients were men and 268 (58.64 %) were women; the male:female ratio was 1:1.42, but in patients aged <21 years, the proportion of male cases was higher than that of females. The average age was 41.2 years (range: 3 months to 87 years). A total of 86 cases were children (≤14 years), which accounted for 18.82 % of all patients. A total of 434 (94.97 %) patients lived in rural areas where the lives and jobs were usually associated with vegetation material and soil contact. The onset of 126 cases (27.57 %) was in spring, 65 (14.22 %) in summer,

83 (18.16 %) in autumn, and 183 (40.04 %) in winter. The onset of disease was related to prior trauma in 133 cases (29.1 %). All the patients denied close contact with animals, sporotrichosis patients, or suspect sporotrichosis patients within 3 months before the onset of the symptoms (Song et al. 2013). More recently, the molecular phylogeny of 64 clinical isolates that were initially identified as *S. schenckii* was investigated. All of the isolates were clustered in a distinct clade with a type of *S. globosa*. According to the authors of these studies, *S. globosa* is the causal agent of the tested sporotrichosis in China, rather than *S. schenckii* (Liu et al. 2014).

In India, sporotrichosis is known to be endemic in the sub-Himalayan regions ranging from Himachal Pradesh in the north-west to Assam and West Bengal in the east (Mehta et al. 2007). It has also been reported sporadically in other states including Punjab, Delhi, Uttar Pradesh, Bihar, Tripura, Meghalaya Andhra Pradesh, Chennai, Karnataka, and Kerala (Randhawa et al. 2003). In 2006, Devi et al. published a retrospective analysis of all cases of sporotrichosis to examine the pattern and frequency of sporotrichosis cases in Manipur over a period of 6 years from July 1999 to June 2005. A total of 73 cases of sporotrichosis were detected, 30 of which were confirmed by culture and 43 of which were diagnosed by aspiration cytology only. Most of the patients were aged 21–40 years ($n = 23$; 31.5 %); there were 39 females (53.4 %) and 34 males (46.5 %). The most common site of infection was the upper limbs ($n = 39$; 53.4 %) followed by the lower limbs ($n = 17$; 23.2 %). The most common type of infection was LS ($n = 46$; 63.1 %) followed by FCS ($n = 27$; 36.9 %). Among these three cases, two male patients (2.7 %) were found to be HIV positive. In this experience, collection of material by aspiration of pus or infected tissue was found to be a better method than scraping or exudate. The authors concluded that Manipur is a new endemic area for sporotrichosis in India (Devi et al. 2006).

Agarwal et al., in 2008, reported the first series of cutaneous sporotrichosis in Uttarakhand, a state situated in the north-western region of India (Agarwal et al. 2008). More recently, three autochthonous cases of LS from east and south districts of Sikkim were reported. Sikkim is a small Himalayan state situated 27.330N; 88.620E in the eastern Himalayas, spread below the world's third highest mountain, the Kanchendzonga. Climatic conditions range from sub-tropical in the foothills to temperate as altitude increases. All three patients had no history of travelling outside the state and did not own pets or domestic animals; thus, the chance of acquiring the disease from the neighboring endemic state of West Bengal or from a zoonotic source was low. These patients had obvious histories of injury that made them vulnerable to the infectious propagules of *S. schenckii*. Awareness of this disease and an extensive environmental study is required to understand the actual burden of this disease (Bhutia et al. 2011).

Other cases have been reported in Asia and Pacific islands, including Korea (Houh et al. 1995; Ishizaki et al. 2004; Lee et al. 2015), Laos (Newton et al. 2005), Indonesia (Campbell 1965), Thailand (Kwangsukstith et al. 1990), and New Zealand (Black et al 1968).

1.2.7 Australia

The first case of sporotrichosis in Australia was reported in 1951 in an older Sydney gardener (Robinson and Orban 1951). Several fungal isolations from the Sydney area were subsequently reported (Muir and Pritchard 1984). In 1998, Conias and Wilson reported an epidemic of sporotrichosis in a south-east Queensland rural community (Conias and Wilson 1998). A total of 16 cases of cutaneous sporotrichosis were seen over a 9-month period in the Darling Downs region of Queensland. All patients had had contact with a batch of moldy hay presumed contaminated by *S. schenckii*. Nine of the 16 patients were male; the youngest patient was 11 and the oldest was 67 years old. LS was seen in 50 % of patients; the rest demonstrated FCS. No cases of disseminated cutaneous or systemic sporotrichosis were seen. One case demonstrated lymphangitis related to sporotrichosis. No apparent difference in the duration to diagnosis was demonstrated between LC or FCS types.

The Path West Laboratory at the QEII Medical Centre, which has branches throughout metropolitan and regional Western Australia, reported 41 microbiologically confirmed human cases from 2000 to 2003 compared with eight cases from 1997 to 1999. Feeney et al. (2007) reviewed these cases and found that 22 were from the Busselton-Margaret River (BMR) region of Western Australia, where no cases had previously been recorded. Epidemiologic investigation and mycologic culture positive for *S. schenckii* implicated hay initially distributed through a commercial hay supplier as the source of the outbreak. Declining infection rates have occurred after various community measures were instigated. Information about diagnosis and management of the infection was distributed to general practitioners in the area, and general information was distributed to the community through various sources such as community newspapers. Since the initial outbreak of sporotrichosis in the BMR region, the infection rate has decreased.

More recently, the geographical, epidemiological, and clinical features of sporotrichosis in the New South Wales (NSW) mid-north coast were determined. The authors conducted a retrospective case review of *S. schenckii* infections occurring during the period 2000–2010. They identified 31 cases of *S. schenckii* infection and concluded that the epidemiological pattern in NSW appears most consistent with sporadic occurrence in an endemic setting (Sivagnanam et al. 2012). Finally, a cluster of six cases was identified in the NSW mid-north coast area in the first half of 2013. All cases were exposed to moldy or contaminated hay (Dhingra et al 2015).

1.3 Conclusion and Future Perspectives

Sporotrichosis is an emergent invasive mycosis that causes skin and subcutaneous infection and, more rarely, disseminated infection in immunosuppressed patients. Cases have been reported throughout the world, but it is most common in tropical and subtropical developing countries. Sporotrichosis can be also diagnosed in

domestic and wild mammals. In veterinary medicine, it is most frequently seen in cats and horses. Cats can have a particularly severe form of cutaneous sporotrichosis and can also serve as a source of zoonotic infection to individuals who handle them and are exposed to exudate skin lesions. There is evidence of species variation and disease manifestation according to geographic location, but the relevance of this association remains an important and unanswered question. Nowadays, sporotrichosis is less a neglected disease, instead becoming a public health concern in many countries.

References

Addy JH (1992) Disseminated cutaneous sporotrichosis associated with anergic immunosuppression due to miliary tuberculosis. West Afr J Med 11(3):216–220

Agarwal S, Gopal K, Umesh, Kumar B et al (2008) Sporotrichosis in Uttarakhand (India): a report of nine cases. Int J Dermatol 47(4):367–371

Ajello L, Kaplan W (1969) A new variant of *Sporothrix schenckii*. Mykosen 12(11):633–644

Alberici F, Paties CT, Lombardi G, Ajello L et al (1989) *Sporothrix schenckii* var lurei as the cause of sporotrichosis in Italy. Eur J Epidemiol 5(2):173–177

Arenas R, Miller D, Campos-Macias P (2007) Epidemiological data and molecular characterization (mtDNA) of *Sporothrix schenckii* in 13 cases from Mexico. Int J Dermatol 46(2):177–179

Arrillaga-Moncrieff I, Capilla J, Mayayo E, Marimon R et al (2009) Different virulence levels of the species of *Sporothrix* in a murine model. Clin Microbiol Infect 15:651–655

Azogue E (1981) Esporotricosis cutanea en el departamento de Santa Cruz-Bolivia/Esporotricosis skin in the district Santa Cruz Cruz Bolivia. Bol Inf Cenetrop 7(1):48–52

Barile F, Mastrolonardo M, Loconsole F, Rantuccio F (1993) Cutaneous sporotrichosis in the period 1978–1992 in the province of Bari, Apulia, Southern Italy. Mycoses 5–6:181–185

Barros MB, Schubach TM, Galhardo MC, Schubach AO et al (2001) Sporotrichosis: na emergent zoonosis in Rio de Janeiro. Mem Inst Oswaldo Cruz 96:777–779

Barros MB, Schubach Ade O, do Valle AC et al (2004) Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: Description of a series of cases. Clin Infect Dis 38:529–535

Barros MB, Schubach AO, Schubach TM, Wanke B et al (2008) An epidemic of sporotrichosis in Rio de Janeiro, Brazil: epidemiological aspects of a series of cases. Epidemiol Infect 136:1192–1196

Betancourt O, Zaror L, Senn C (2013) Aislamiento de hongos filamentosos desde pelaje de gatos sin lesiones dermicas en Temuco, Chile. Revista Cientifica de la Facultad de Ciencias Veterinarias 23(5):380

Bhutia PY, Gurung S, Yegneswaran PP, Pradhan J et al (2011) A case series and review of sporotrichosis in Sikkim. J Infect Dev Ctries 5(8):603–608

Black H, Taylor DE, Yeoman DM (1968) Sporotrichosis in New Zealand: a case report. N Z Med J 68(434):27–30

Bonifaz A, Saúl A, Paredes-Solis V, Fierro L et al (2007) Sporotrichosis in childhood: clinical and therapeutic experience in 25 patients. Pediatr Dermatol 24(4):369–372

Borges TS, Rossi CN, Fedullo JD et al (2013) Isolation of *Sporothrix schenckii* from the claws of domestic cats (indoor and outdoor) and in captivity in São Paulo (Brazil). Mycopathologia 176:129–137

Brown R, Weintroub D, Simpson M W (1947) Timber as a source of sporotrichosis infection. In: Proceedings of the Transvaal Mine Medical Officers' Association, Johanesburg pp. 5–38

Bunce PE, Yang L, Chun S, Zhang SX et al (2012) Disseminated sporotrichosis in a patient with hairy cell leukemia treated with amphotericin B and posaconazole. Med Mycol 50:197–201

Bustamante B, Campos PE (2001) Endemic sporotrichosis. Curr Opin Infect Dis 14(2):145–149
Cafarchia C, Sasanelli M, Lia RP et al (2007) Lymphocutaneous and nasal sporotrichosis in a dog from Southern Italy: case report. Mycopathologia 163:75–79
Calero C, Tapia A (1962) Two cases of sporotrichosis in the Isthmus of Panama. Am J Trop Med Hyg 11:676–677
Callens SF, Kitetele F, Lukun P et al (2006) Pulmonary *Sporothrix schenckii* infection in a HIV positive child. J Trop Pediatr 52(2):144–146
Camacho E, León-Navarro I, Rodríguez-Brito S et al (2015) Molecular epidemiology of human sporotrichosis in Venezuela reveals high frequency of *Sporothrix globosa*. BMC Infect Dis 15 (1):94
Campbell C (1965) A case of sporotrichosis occurring in Papua. Med J Aust 2(1):23
Carmichael JW (1962) Chrysosporium and some other aleuriosporic hyphomycetes. Can J Bot 40:1137–1173
Carod JF, Ramarozatovo L, Randrianasolo P et al (2007) Cutaneous sporotricosis in a Malagasy patient. Med Trop (Mars) 67(1):18
Carr MM, Fielding JC, Sibbald G et al (1995) Sporotrichosis of the hand: an urban experience. J Hand Surg [Am] 20(1):66–70
Civila ES, Bonasse J, Conti-Díaz IA et al (2004) Importance of the direct fresh examination in the diagnosis of cutaneous sporotrichosis. Inter J Dermatol 43:808–810
Coles FB, Schuchat A, Hibbs JR et al (1992) A multistate outbreak of sporotrichosis associated with sphagnum moss. Am J Epidemiol 136:475–487
Conias S, Wilson P (1998) Epidemic cutaneous sporotrichosis: report of 16 cases in Queensland due to mouldy hay. Australas J Dermatol 39:34–37
Conti Díaz IA (1989) Epidemiology of sporotrichosis in Latin America. Mycopathologia 108:113–116
Criseo G, Romeo O (2010) Ribosomal DNA sequencing and phylogenetic analysis of environmental *Sporothrix schenckii* strains: comparison with clinical isolates. Mycopathologia 169:351–358
Criseo G, Malara G, Romeo O et al (2008) Lymphocutaneous sporotrichosis in an immunocompetent patient: a case report from extreme southern Italy. Mycopathologia 166(3):159–162
Cruz R, Vieille P, Oschilewski D (2012) *Sporothrix globosa* isolation related to a case of lymphocutaneous sporotrichosis. Rev Chil Infectol 29(4):401–405
D'Alessio DJ, Leavens LJ, Strumpf GB et al (1965) An outbreak of sporotrichosis in Vermont associated with sphagnum moss as the source of infection. N Engl J Med 20(272):1054–1058
Dahl BA, Silberfarb PM, Sarosi GA et al (1971) Sporotrichosis in children. Report of an epidemic. JAMA 215:1980–1982
Dalis JS, Kazeem HM, Kwaga JK et al (2014) Severe generalized skin lesions due to mixed infection with *Sporothrix schenckii* and *Dermatophilus congolensisin* a bull from Jos Nigeria. Vet Microbiol 172(3-4):475–478
de Beer ZW, Harrington TC, Vismer HF et al (2003) Phylogeny of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 95(3):434–441
de Beurmann L, Gougerot H (1912) Les sporotrichoses. Felix Alcan, Paris
de Hoog GS (1974) The genera Blastobotrys, Sporothrix, Calcarisporium and Calcarisporiella gen nov. Stud Mycol 7:1–119
de Hoog GS, Guarro J, Gené J et al (2000) Atlas of clinical fungi, 2nd edn. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands
de Lezcano LB, Di Martino B, Villalba J, Rodríguez M et al (2008) An unusual clinical presentation of fixed cutaneous sporotrichosis. Report of two cases. Rev Panam Infectol 10 (3):45–47
de Meyer EM, de Beer ZW, Summerbell RC et al (2008) Taxonomy and phylogeny of new wood- and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 100:647–661

Devi KR, Devi MU, Singh TN, Devi KS et al (2006) Emergence of sporotrichosis in Manipur. Indian J Med Microbiol 24(3):216–219
Dhingra D, Durrheim D, Porigneaux P (2015) Sporotrichosis outbreak and mould hay in NSW. Aust Fam Physician 44(4):217–221
Dias NM, Oliveira MME, Portela MA et al (2011) (2011) Sporotrichosis caused by *Sporothrix mexicana*. Portugal Emerg Infec Dis 17:1975–1976
Dixon DM, Salkin IF, Duncan RA et al (1991) Isolation and characterization of *Sporothrix schenckii* from clinical and environmental sources associated with the largest U.S epidemic of sporotrichosis. J Clin Microbiol 29(6):1106–1113
Dooley DP, Bostic PS, Beckius ML (1997) Spook house sporotrichosis. A point-source outbreak of sporotrichosis associated with hay bale props in a Halloween haunted-house. Arch Intern Med 157:1885–1887
Dunstan RW, Langham RF, Reimann KA et al (1986) Feline sporotrichosis: a report of five cases with transmission to humans. J Am Acad Dermatol 15(1):37–45
El-Mofty AM, Nada M (1965) Sporotrichosis in Egypt. Br J Dermatol 77:357–364
Epinosa-Texis A, Hernández-Hernandez F, Lavalle P et al (2001) Study of 50 patients with sporotrichosis. Clinical and laboratory assessment. Gac Med Mex 137(2):111–116
Escaffi F, María José Benedetto B, Antonieta Podlipnik C, Sebastián (2010) Esporotricosis cutánea: revisión a propósito de un caso contraído en Chile/Cutaneous sporotrichosis: a Chilean case report. Rev Chil Dermatol 26(2):154–158
Feeney KT, Arthur IH, Whittle AJ, Altman SA et al (2007) Outbreak of sporotrichosis, Western Australia. Emerg Infec Dis 13:1228–1231
Fernandes GF, dos Santos PO, Rodrigues AM et al (2013) Characterization of virulence profile, protein secretion and immunogenicity of different *Sporothrix schenckii* sensu stricto isolates compared with *S. globosa* and *S. brasiliensis* species. Virulence 4(3):1–9
Fernández-Silva F, Capilla J, Mayayo E et al (2012) Virulence of *Sporothrix luriei* in a murine model of disseminated infection. Mycopathologia 173(4):245–249
Fleury RN, Taborda PR, Gupta AK et al (2001) Zoonotic sporotrichosis. Transmission to humans by infected domestic cat scratching: report of four cases in São Paulo. Brazil Int J Dermatol 40 (5):318–322
Flores C, Ferrada L, De la Parra M (1982) Estudio micológico y experimental de un caso de esporotricosis. Bol Micol 1:39–42
Freitas D, Migliano M, Zani Neto L (1956) Esporotricose—Observação de caso espontâneo em gato doméstico (F. catus). Rev Fac Med Vet Univ São Paulo 5(4):601–604
Freitas D, Moreno G, Saliba A et al (1965) Esporotricose em cães e gatos. Rev Fac Med Vet Univ São Paulo 7(2):381–387
Freitas DF, de Siqueira HB, do Valle AC et al (2012) Sporotrichosis in HIV-infected patients: report of 21 cases of endemic sporotrichosis in Rio de Janeiro, Brazil. Med Mycol 50:170–178
Fujii H, Tanioka M, Yonezawa M et al (2008) A case of atypical sporotrichosis with multifocal cutaneous ulcers. Clin Exp Dermatol 33(2):135–138
Ghosh A, Chakrabarti A, Sharma VK et al (1999) Sporotrichosis in Himachal Pradesh (North India). Trans R Soc Trop Med Hyg 93:41–45
Gori S, Lupetti A, Moscato G et al (1997) Pulmonary sporotrichosis with hyphae in a human immunodeficiency virus-infected patient. A case report. Acta Cytol 41(2):519–521
Gremião ID, Menezes RC, Schubach TM et al (2015) Feline sporotrichosis: epidemiological and clinical aspects. Med Mycol 53(1):15–21
Gumaa SA (1978) Sporotrichosis in Sudan. Trans R Soc Trop Med Hyg 72(6):637–640
Gurcan S, Konuk E, Kilic H et al (2007) Sporotrichosis, a disease rarely reported from Turkey, and an overview of Turkish literature. Mycoses 50:426–429
Hajjeh R, McDonnell S, Reef S et al (1997) Outbreak of sporotrichosis among tree nursery workers. J Infect Dis 176:499–504
Hawks GH (1955) Sporotrichosis Report of tree cases from Toronto. Can Med Assoc J 72:28–32

Hektoen L, Perkins CF (1900) Refractory subcutaneous abscesses caused by *Sporothrix schenckii*. A new pathogenic fungus. J Exp Med 5:77–89
Houh W, Chung SR, Ro BI (1995) Itraconazole in the treatment of sporotrichosis: a Korean experience. Trop Doct 25(3):107–109
Howard DH (1961) Dimorphism of *Sporotrichum schenckii*. J Bacteriol 81:464–469
Inokuma D, Shibaki A, Shimizu H (2010) Two cases of cutaneous sporotrichosis in continental/microthermal climate zone: global warming alert? Clin Exp Dermatol 35:668–676
Ishizaki H, Kawasaki M, Aoki M et al (2004) Mitochondrial DNA analysis of *Sporothrix schenckii* from China, Korea and Spain. Nihon Ishinkin Gakkai Zasshi 45(1):23–25
Jacyk WK, Lawande RV, Tulpule SS (1981) Deep mycoses in West Africa: a report of 13 cases and review of the Nigerian literature. J Natl Med Assoc 73(3):251–256
Jirásek L, Fragner P, Pavlásková I (1976) A case of epidermal sporotrichosis. Cesk Dermatol 51 (3):162–167
Kazemi A, Razi A (2007) Sporotrichosis in Iran. Rev Iberoam Micol 24:38–40
Kong X, Xiao T, Lin J et al (2006) Relationships among genotypes, virulence and clinical forms of *Sporothrix schenckii* infection. Clin Microbiol Infect 12(11):1077–1081
Krempl-Lamprecht L (1978) Tropic-subtropic fungus infections in Germany. Hautarzt 29 (1):17–21
Kusuhara M, Hachisuka H, Sasai Y (1988) Statistical survey of 150 cases with sporotrichosis. Mycopathologia 102:129–133
Kwangsukstith C, Vanittanakom N, Khanjanasthiti P et al (1990) Cutaneous sporotrichosis in Thailand: first reported case. Mycoses 33(11-12):513–517
Laur WE, Posey RE, Waller JD (1979) A familial epidemic of cutaneous sporotrichosis occurring in north Texas. Cutis 23:205–208
Learmonth GE (1915) Observations on a Sporothrix bearing some resemblance to the *Sporothrix beurmanni*. Can Med Assoc J 5(1):32–38
Lee H, Kim DY, Lee KH (2015) Deformity of the earlobe caused by fixed cutaneous sporotrichosis in a pediatric patient. Int J Dermatol 54(5):e187–e189
Liu TT, Zhang K, Zhou X (2014) Molecular identification of Sporothrix clinical isolates in China. J Zhejiang Univ Sci B 15(1):100–108
Lutz A, Splendore A (1907) On a mycosis observed in men and mice: Contribution to the knowledge of the so-called sporotrichosis. Revista Médica de São Paulo 21:443–450
Lyon GM, Zurita S, Casquero J et al (2003) Population-based surveillance and a case-control study of risk factors for endemic lymphocutaneous sporotrichosis in Peru. Clin Infec Dis 36:34–39
Mackinnon JE (1972) Regional peculiarities of some deep mycoses. Mycopathol Mycol Appl 46:249–265
Madrid H, Cano J, Gene J et al (2009) *Sporothrix globosa*, a pathogenic fungus with widespread geographical distribution. Rev Iberoam Micol 26(3):218–222
Madrid H, Gené J, Cano J et al (2010) *Sporothrix brunneoviolacea* and *Sporothrix dimorphospora*, two new members of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 102:1193–1203
Madrid IM, Mattei AS, Fernandes CG et al (2012) Epidemiological Findings and Laboratory Evaluation of Sporotrichosis: A Description of 103 Cases in Cats and Dogs in Southern Brazil. Mycopathologia 173:265–273
Magand F, Perrot JL, Cambazard F et al (2009) Sporotrichose cutanée autochtone française. Ann Dermatol Venereol 2009(136):273–275
Mahajan VK, Sharma NL, Sharma RC et al (2005) Cutaneous sporotrichosis in Himachal Pradesh, India. Mycoses 48:25–31
Marimon R, Gené J, Cano J et al (2006) Molecular phylogeny of *Sporothrix schenckii*. J Clin Microbiol 44:3251–3256
Marimon R, Cano J, Gené J et al (2007) *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol 45:3198–3206

Marimon R, Gené J, Cano J et al (2008) *Sporothrix luriei*: a rare fungus from clinical origin. Med Mycol 46:621–625
Mata-Essayag S, Delgado A, Maria T, Colella MT et al (2013) Epidemiology of sporotrichosis in Venezuela. Int J Dermatol 52:974–980
Matter SE, Bailey DM, Sexton DJ (1984) Immune deficiency presenting as disseminated sporotrichosis. J Okla State Med Assoc 77:114–117
Mayorga R, Cáceres A, Toriello et al (1979) Investigación de uma zona endemica de esporotricosis em La region de La Laguna de Ayarza, Guatemala. Bol Sanit Panam 87 (1):20–34
Mayorga-Rodriguez JA, Barba-Rubio J, Munõz-Estrada VF et al (1997) Sporotrichosis in Jalisco state: a clinical epidemiological study (1960±1996). Dermatol Rev Mex 41:105–108
McCarty TP, Pappas PG (2014) The evolving epidemiology of sporotrichosis. Curr Fungal Infect Rep 8(4):255–261
Mehta KI, Sharma NL, Kanga AK et al (2007) Isolation of *Sporothrix schenckii* from the environmental sources of cutaneous sporotrichosis patients in Himachal Pradesh, India: results of a pilot study. Mycoses 50(6):496–501
Mesa-Arango AC, Del Rocío R-MM, Pérez-Mejía A et al (2002) Phenotyping and genotyping of *Sporothrix schenckii* isolates according to geographic origin and clinical form of Sporotrichosis. J Clin Microbiol 40(8):3004–3011
Mora-Cabrera M, Alonso RA, Ulloa-Arvizu R et al (2001) Analysis of restriction profiles of mitochondrial DNA from *Sporothrix schenckii*. Med Mycol 39:439–444
Morgado FN, Schubach AO, Barros MB et al (2011) The in situ inflammatory profile of lymphocutaneous and fixed forms of human sporotrichosis. Med Mycol 49(6):612–620
Muir DB, Pritchard RC (1984) *Sporothrix schenckii*—incidence in the Sydney region. Australas J Dermatol 25:27–28
Nakamura Y, Sato H, Watanabe S et al (1996) *Sporothrix schenckii* isolated from a cat in Japan. Mycoses 39(3-4):125–128
Negroni R, Maiolo E, Arechavala AI et al (2007) Problemas clínicos em Micologia Medica: problema n° 25. Rev Iberoam Micol 24:79–81
Newton PN, Chung WH, Phetsouvanh R et al (2005) Sporotrichosis, Plain of Jars, Lao People's Democratic Republic. Emerg Infect Dis 11(9):1496–1497
Neyra E, Fonteyne PA, Swinne D et al (2005) Epidemiology of human sporotrichosis investigated by amplified fragment length polymorphism. J Clin Microbiol 43(3):1348–1352
Nusbaum BP, Nita Gulbas N, Horwitz N (1983) Sporotrichosis acquired from a cat. J Am Acad Dermatol 8:386–391
Oda S, Kotani Y, Matsumura T (1964) Two cases of sporotrichosis in mie prefecture. Hifuka Kiyo 59:195–199
Ojeda T, Rodríguez-Pichardo A, Suárez AI (2011) Esporotricosis en la provincia de Sevilla (España) Carta científica. Enferm Infec Microbiol Clin 29(3):232–242
Oliveira MME, Almeida-Paes R, Muniz MM et al (2011) Phenotypic and molecular identification of *Sporothrix* isolates from an epidemic area of sporotrichosis in Brazil. Mycopathologia 172:257–267
Oliveira MM, Almeida-Paes R, Gutierrez-Galhardo MC et al (2014) Molecular identification of the *Sporothrix schenckii* complex. Rev Iberoam Micol 31(1):2–6
Pappas PG, Tellez I, Deep AE et al (2000) Sporotrichosis in Peru: Description of an Area of Hyperendemicity. Clin Infect Dis 30:65–70
Pardo-Castello V, Trespalacios F (1959) Superficial and deep mycoses in Cuba: a report based on 1,174 cases. South Med J 52(1):7–15
Park CH, Greer CL, Cook CB (1972) Cutaneous sporotrichosis: recent appearance in Northern Virginia. Am J Clin Pathol 57(1):23–26
Pereira SA, Menezes RC, Gremião ID et al (2011) Sensitivity of cytopathological examination in the diagnosis of feline sporotrichosis. J Feline Med Surg 13:220–223

Pereira SA, Gremião ID, Kitada AA et al (2014) The epidemiologicals cenário of feline sporotrichosis in Rio de Janeiro, Stateof Rio de Janeiro, Brazil. Rev Soc Bras Med Trop 47(3):392–393

Pérez-Fernández N, Jaka-Moreno A, de la Cabac I et al (2013) Nodular skin lesions in a patient from Nicaragua. Enferm Infecc Microbiol Clin 31(2):114–115

Pérez-Morales L, Iglesias-López M, Quiñones-Cherta O et al (2014) Aislamiento microbiológico de *Sporothrix schenckii* en un paciente inmunodeprimido. Presentación de un caso. Medisur 12 (4):662–669

Pönnighaus M, Grosser S, Baum HP (2003) Sporotrichosis as the cause of a leg ulcer. Hautarzt 54 (1):64–66

Powell KE, Taylor A, Phillips BJ et al (1978) Cutaneous sporotrichosis in forestry workers: epidemic due to contaminated sphagnum moss. JAMA 240:232–235

Ramírez M, Andagua J, Lizárraga J et al (2011) Esporotricosis en pacientes que acuden a un centro medico de referencia en Abancay, Perú. Rev Peru Med Exp Salud Publica 28:508–512

Randhawa HS, Chand R, Mussa AY et al (2003) Sporotrichosis in India: First case in a Delhi resident and an update. Indian J Med Microbiol 21:12–16

Rippon JW (1988) Sporotrichosis. In: Rippon JW (ed) Medical Mycology. The Pathogenic Fungi and Pathogenic Actinomycetes, 3rd edn. WB Saunders, Philadelphia PA, pp 325–352

Robinson CF, Orban TD (1951) A case of regional lymphatic sporotrichosis. Aust J Dermatol 1:142–144

Rodrigues AM, Teixeira MM, Hoog G et al (2013a) Phylogenetic Analysis Reveals a High Prevalence of *Sporothrix brasiliensis* in Feline Sporotrichosis Outbreaks. PLoS Negl Trop Dis 7(6):1–14

Rodrigues AM, de Hoog GS, Camargo ZP (2013b) Emergence of pathogenicity in the *Sporothrix schenckii* complex. Med Mycol 51(4):405–412

Rodrigues AM, de Hoog GS, Zhang Y et al (2014) Emerging sporotrichosis is driven by clonal and recombinant *Sporothrix* species. Emerg Microbes Infect 3, e32

Roets F, de Beer ZW, Wingfield MJ et al (2008) *Ophiostoma gemellus* and *Sporothrix variecibatus* from mites infesting Protea in fructescences in South Africa. Mycologia 100:496–510

Romero-Cabello R, Bonifaz A, Romero-Feregrino R et al (2011) Disseminated sporotrichosis BMJ Case Rep 25 doi: 10.1136/bcr.10.2010.3404

Sánchez-Saldaña L, Galarza CM, Matos-Sánchez R (2009) Infecciones micóticas subcutáneas. Dermatología Peruana 19(4):362–387

Schenck BR (1898) Refractory subcutaneous abscesses caused by a fungus possibly related to the sporotricha. Bull Johns Hopkins Hosp 9:286–290

Schubach TM, de Oliveira SA, dos Reis RS et al (2002) *Sporothrix schenckii* isolated from domestic cats with and without sporotrichosis in Rio de Janeiro, Brazil. Mycopathologia 153:83–86

Schubach TM, Schubach A, Okamoto T et al (2004) Evaluation of an epidemic of sporotrichosis in cats: 347 cases (1998-2001). J Am Vet Med Assoc 224:1623–1629

Silva MBT, Costa MMM, Torres CCS (2012) Urban sporotrichosis: a neglected epidemic in Rio de Janeiro, Brazil. Cad Saúde Pública Rio de Janeiro 28(10):1867

Sivagnanam S, Bannan AM, Chen SC et al (2012) Sporotrichosis (*Sporothrix schenckii* infection) in the New South Wales mid-north coast, 2000–2010. Med J 196:588–590

Song Y, Li SS, Zhong SX et al (2013) Report of 457 sporotrichosis cases from Jilin province, northeast China, a serious endemic region. J Eur Acad Dermatol Venereol 27:313–318

Souza LL, Nascente PS, Nobre MO et al (2006) Isolation of *Sporothrix schenckii* from the nails of healthy cats. Braz J Microbiol 37:372–374

Staib F, Grosse G, Male O et al (1974) Utilization of creatinine and guanidino-acetic acid by *Sporothrix schenckii*. Microbiological epidemiological and pathologico-anatomical studies on the vector of sporotrichosis in Austria. Z Hautkr 49(14):607–613

Symmers WS (1968) Sporotrichosis in Ireland. Ulster Med J 37(2):85–101

Takenaka M, Yoshizaki A, Utani A et al (2014) A survey of 165 sporotrichosis cases examined in Nagasaki prefecture from 1951 to 2012. Mycoses 57:294–298

Travassos LR, Lloyd KO (1980) *Sporothrix schenckii* and related species of *Ceratocystis*. Microbiol Rev 44:683–721

Vidal G, Rodríguez de Kop N (1993) Esporotricosis: enfoque epidemiológico, clínico y terapêutico/Sporotrichosis: clinical, therapeutic and epidemiologic overview. Arch Argent Dermatol 43(4):221–234

Vismer HF, Hull PR (1997) Prevalence, epidemiology, and geographical distribution of *Sporothrix schenckii* infections in Gauteng, South Africa. Mycopathologia 137(3):137–143

Wroblewska M, Swoboda-Kopec E, Kawecki D et al (2005) Infection by a dimorphic fungus *Sporothrix schenckii* in an immune compromised patient. Infection 33(4):289–291

Zhang MF, Zhang YQ, Jiang P et al (1996) Isolation of *Sporothrix schenckii* from Reed. Chin J Dermatol 29:322–333

Zhang YQ, Xu XG, Zhang M et al (2011) Sporotrichosis: clinical and histopathological manifestations. Am J Dermatopathol 33:296–302

Zurita MS (2014) Esporotricosis y paracoccidioidomicosis en Perú: experiencias en prevención y control. Rev Peru Med Exp Salud Publica 31(2):352–357

Chapter 2
Sporothrix schenckii Complex: Genetic Polymorphism

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Abstract *Sporothrix schenckii* is an ascomycetous dimorphic fungus that, for over a century, was recognized as the sole agent of sporotrichosis, a subcutaneous mycosis with a worldwide distribution. Based on physiologic and molecular aspects, however, it has been proposed that *S. schenckii* is a complex of distinct species: *S. brasiliensis*, *S. mexicana*, *S. globosa*, *S. schenckii sensu stricto*, *S. luriei*, and *S. pallida* (formerly *S. albicans*). Before the description of these species, several authors had pointed to a high level of genetic diversity among *Sporothrix* spp. strains according to geographic distribution and clinical forms. Phenotypic characterization is usually made through morphology of colony and conidia and biochemical profiles. However, the correlation between molecular data and phenotypic characteristics is fundamental to the identification of the *Sporothrix* complex. Molecular information about the *Sporothrix* species complex is scarce. Until now, *S. brasiliensis* and *S. schenckii s. str.* are the only clinically relevant species of this complex with an elucidated genome sequence, thus limiting molecular knowledge about the cryptic species of this complex, the population structure, and the sexual form of all *S. schenckii* complex species. In this chapter, we focus on the current molecular tools applied to the identification of the *Sporothrix* complex species and on published studies on *Sporothrix* spp. sexuality, and we outline the geographic distribution of *Sporothrix* complex species.

Keywords *Sporothrix schenckii* • Polymorphism • Genetic variability

2.1 Introduction

Sporothrix schenckii is an ascomycetous dimorphic organism (Ascomycota, Pyrenomycetes, Ophiostomatales, Ophiostomataceae) that is found in substrates such as living and decayed vegetation, animal excreta, and soil (Barros et al. 2011;

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Guarro 2012). This fungus is phenotypically characterized by the ability to produce sessile dematiaceous conidia along with hyaline sympodial conidia in its filamentous form, and cigar-shaped yeast-like cells in parasitism or when cultured at 35–37 °C on specific culture media (Barros et al. 2011; Marimon et al. 2007).

For over a century, this species was recognized as the sole agent of sporotrichosis, a subcutaneous mycosis with a worldwide distribution (Lopes-Bezerra et al. 2006). Until 2007, *S. schenckii* was considered a single taxon, although Liu et al. (2003) previously reported the existence of high genetic variation within this species by means of molecular polymorphisms detected by the random amplification of polymorphic DNA (RAPD) technique that were related to the geographical origin of strains and clinical manifestations of sporotrichosis. Later, other studies also reported genetic variation within *S. schenckii* after comparative analysis of clinical strains by RAPD and internal transcriber spacer (ITS) sequencing (Gutierrez-Galhardo et al. 2008; Reis et al. 2009).

Nevertheless, it has been proposed, based on physiologic and molecular aspects, that *S. schenckii*, instead of a single taxon, is a complex of four distinct species: *S. brasiliensis*, *S. mexicana*, *S. globosa*, and *S. schenckii sensu stricto* (Marimon et al. 2007). Later, *S. schenckii* var. *luriei* was considered another species belonging to the *S. schenckii* complex, and was therefore named *S. luriei* (Marimon et al. 2008a). Additionally, other phylogenetic analysis with *S. albicans*, *S. pallida*, and *S. nivea* revealed a significant similarity among them. Therefore, it has been proposed that all these three non-pathogenic species closely related to *S. schenckii* be called *S. pallida* (de Meyer et al. 2008; Romeo et al. 2011). In fact, Romeo et al. (2011) studied the molecular phylogeny and epidemiology of *S. schenckii* complex strains isolated in Italy and demonstrated that 26 environmental strains co-clustered with *S. pallida* and two clinical strains grouped with *S. schenckii s. str*. Furthermore, a recent report showed *S. pallida*, recognized so far as an exclusively environmental species, caused keratitis in a corneal transplant recipient (Morrison et al. 2013). Therefore, *S. schenckii s. str*., *S. brasiliensis*, *S. globosa*, and in minor proportions *S. mexicana*, *S. pallida*, and *S. luriei* are now recognized as agents of sporotrichosis.

Moreover, three other environmental *Sporothrix* species were described using molecular approaches: *S. stylites*, *S. humicola*, and *S. lignivora*. The two first species differ from *S. schenckii* by the sole production of hyaline conidia and consequently shows no darkening of colonies with age. *S. lignivora* has distinctive conidia that do not match in size and shape with other *Sporothrix* or *Ophiostoma* species. Isolates classified as *S. humicola* in this study were previously referred to as environmental *S. schenckii* isolates. In their study, the authors conclude that β-tubulin gene sequence analysis is strongly recommended in taxonomic studies from *Sporothrix* species isolated from the environment (de Meyer et al. 2008). In fact, β-tubulin analysis, together with ITS sequencing, enables the further description of two other environmental *Sporothrix* species: *S. brunneoviolacea* and *S. dimorphospora* (Madrid et al. 2010).

2.2 Differences Among the Species of the *Sporothrix* Complex

Cultures of the members of the *Sporothrix* complex in mycological media such as Sabouraud dextrose agar, potato dextrose agar, or mycobiotic agar yield white filamentous colonies that become brown to black after a few days. Subculturing these colonies in brain heart infusion at 35–37 °C results in white to creamy yeast-like colonies (Barros et al. 2011). Identification of the *Sporothrix* complex is based on the macro and micromorphologies of the mycelial and yeast forms. However, these characteristics do not differentiate the newly described species of the *Sporothrix* complex. In order to physiologically differentiate the species within this complex, other tests such as carbohydrate assimilation (especially sucrose and raffinose), growth rates at 30 °C and 37 °C, as well as production of dematiaceous conidia are necessary (Marimon et al. 2008a). Moreover, the species present variability in several gene sequences, and the partial calmodulin gene sequencing (Marimon et al. 2007) is broadly used to differentiate the species of the *Sporothrix* complex (Fig. 2.1).

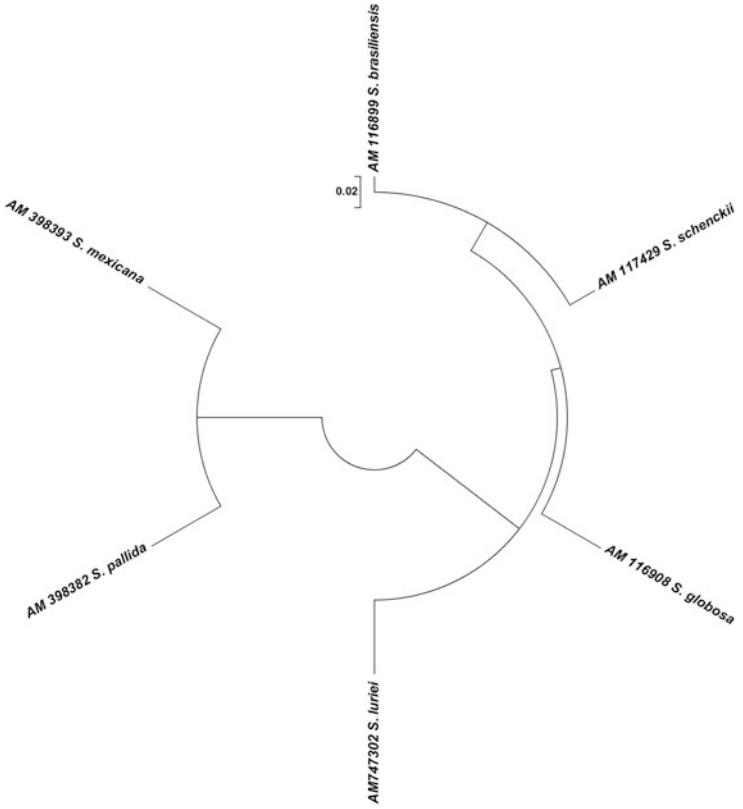

Fig. 2.1 Genetic relationship between the species of the *Sporothrix* complex. Neighbor-Joining tree showing the relatedness of the type strain of each species

Regarding carbohydrate assimilation, *S. schenckii s. str.* and *S. mexicana* are able to assimilate sucrose and raffinose, *S. globosa* and *S. pallida* assimilate only sucrose, and *S. brasiliensis* and *S. luriei* are unable to use any of these two carbohydrates as a sole carbon source for growth. *S. globosa* grows poorly at 37 °C, whereas *S. luriei* grows abundantly at this temperature. Production of dematiaceous conidia is absent in *S. pallida* and *S. luriei*. However, discrepancies between these physiological methods and molecular approaches of identification have been described (Fernandes et al. 2013; Oliveira et al. 2011).

Although geographic limitations are imprecise, epidemiological data indicate that *S. schenckii s. str.* is found predominantly on the American, Asian, and African continents; *S. globosa* has a worldwide distribution with a high frequency in Europe and Asia (Liu et al. 2014; Madrid et al. 2009). *S. brasiliensis* is apparently restricted to Brazil (Marimon et al. 2007; Oliveira et al. 2011; Rodrigues et al. 2013) and *S. mexicana* to Mexican environmental samples (Marimon et al. 2007). However, *S. mexicana* was identified in a case of sporotrichosis in a human patient in Portugal (Dias et al. 2011) and in a re-identification of three clinical isolates maintained in Brazilian fungal collections since 1955 (Rodrigues et al. 2013). *S. luriei* is a very rare pathogen, reported on four human sporotrichosis cases, but isolated only from one case in Africa (Marimon et al. 2008a).

Certain publications have shown that the species of the *Sporothrix* complex differ in virulence and antifungal susceptibility (Arrillaga-Moncrieff et al. 2009; Marimon et al. 2008b). *S. brasiliensis* is described as a highly virulent species, followed by *S. schenckii* and *S. globosa*, which present the lowest virulence among these three species (Arrillaga-Moncrieff et al. 2009). However, the virulence of *S. brasiliensis* does not appear to be related to its antifungal susceptibility, since lower antifungal concentrations are enough to inhibit the growth of this species. On the other hand, *S. mexicana* is highly resistant to most clinically used antifungal drugs, such as fluconazole, itraconazole, voriconazole, ravuconazole, micafungin, and amphotericin B (Marimon et al 2008b).

There are only a few reports regarding the clinical aspects of human sporotrichosis caused by different species of the *Sporothrix* complex. Some publications have suggested that even lesional mechanisms should be related to these different species (Arrillaga-Moncrieff et al. 2009). In general, *S. brasiliensis* is usually associated with unusual clinical manifestations of sporotrichosis such as erythema (Almeida-Paes et al. 2014) or disseminated infection without HIV infection (Orofino-Costa et al. 2013). However, cases of sporotrichosis related to *S. schenckii* (Almeida-Paes et al. 2014) and *S. globosa* (Yu et al. 2013) are closer to the classical description of sporotrichosis cases before reports of the *Sporothrix* complex (Morris-Jones 2002). Therefore, molecular methods that allow rapid identification and differentiation of these closely related fungi are very necessary to the mycology laboratory.

2.3 Sexual Reproduction in the *Sporothrix* Complex: What Is Going On?

Understanding the mechanisms of fungal sexual reproduction is very important since this process strongly influences the level of genetic variability in fungal populations; the study of reproductive biology in *Sporothrix* is crucial because the sexual state of all species of the *S. schenckii* complex is as yet unknown. However, molecular evidence showing that *S. schenckii* undergoes recombination in nature is strong (Mesa-Arango et al. 2002).

Rodrigues et al. (2014a) evaluated the feline host impact on the epidemiology, spatial distribution, prevalence, and genetic diversity of human sporotrichosis and found evidence of recombination in *S. schenckii* but not in *S. brasiliensis*, strongly suggesting that these sibling species follow distinct pathways and strategies during epidemics. The reticulated pattern of *S. schenckii* reported in this study proposes that recombination among genotypes may have contributed to the evolution of divergent strains, and the sexual reproduction in *Sporothrix* is likely to occur in an environmental habitat. However, the feline outbreak genotypes are prevalently clonal, which does not necessarily imply the absence of sex, but does indicate the emergence of a successful genotype.

Molecular analyses from the 18S region of the ribosomal DNA (rDNA) have shown indirect evidence that the *S. schenckii* sexual state was *Ophiostoma stenoceras*. The authors considered that the 18S rDNA gene is highly conserved and may not exhibit sufficient variability to allow a proper distinction between closely related species (Berbee and Taylor 1992). Later, Beer et al. (2003) used ITS sequencing and reported that this anamorph–teleomorph connection was inaccurate. Additionally, morphological and physiological studies showed consistent differences between these two species (Dixon et al. 1991; O'Reilly and Altman 2006). Also, most *Ophiostoma* species have a close association with tree-infesting bark beetles, and some cause tree disease (Suh et al. 2013), and the members of the *Sporothrix* complex are pathogenic fungi that cause human and animal sporotrichosis, with no described cases of plant diseases (Barros et al. 2011). Recently, it has been proposed that the fungus *O. stenoceras* (producing a *Sporothrix* anamorph in culture) is indeed distinct from *S. brasiliensis*, *S. schenckii s. str.*, *S. globosa*, *S. mexicana*, and *S. pallida*, based on calmodulin sequencing (Rodrigues et al. 2013). These results lead us to consider *O. stenoceras* anamorph and *S. schenckii* as distinct species. Meanwhile, molecular studies reinforce that the *Sporothrix* teleomorph belongs to the genus *Ophiostoma*, but that it is different from *O. stenoceras* (Beer et al. 2003; de Meyer et al. 2008; Hintz 1999).

The existence of a sexual state in the *Sporothrix* complex is supported by some studies. In most heterothallic filamentous ascomycete species, the MAT locus bears one of the two idiomorphs, MAT1-1 and MAT1-2, that are required for sexual reproduction and present a low degree of similarity on their gene sequences (Bubnick and Smulian 2007; Casselton 2008). Kano et al. (2013) confirmed the existence of the *MAT1-2* (HMG) gene in *S. globosa* through analysis of its genomic DNA.

The relationships between the *MAT1-2* gene of *S. globosa* and three different species of the genus *Ophiostoma* were also analysed, showing a clear distinction between them, with the four species separated into four clusters. However, a certain degree of homology was noted between the *MAT1-2* gene of *S. globosa* and those of *Ophiostoma* species. The characterization of the partial *MAT1-1* idiomorph (*MAT1-1-1*) of *S. globosa* was performed using the genome walking approach. The *MAT1-1*: *MAT1-2* ratio was also determined on 20 *S. globosa* isolates from clinical cases in Japan. In this study, the authors sequenced *MAT1-1-1* from isolates of *S. schenckii* and *S. globosa*, and the phylogenetic analyses indicated that 92 % of the *MAT1-1-1* sequences between the two species are similar, but they differed from *O. montium*. The intraspecific variation of *MAT1-1-1* was low among isolates of *S. globosa* from different areas in Japan (Kano et al. 2014). Further analysis of the *MAT1-1* gene of members of the *S. schenckii* complex is required, both for its phylogenetic classification as well as for the discovery of teleomorphs of all species in this cryptic complex.

2.4 Genetic Polymorphisms in the *Sporothrix* Complex

Despite the importance of sporotrichosis as a disease with important reported epidemic areas in the last years, just a few studies deal with genetic polymorphisms and genomic architecture of strains of the *Sporothrix* complex. Some publications suggest that molecular polymorphisms of *S. schenckii* can be linked with fungal virulence. A study revealed that a *S. schenckii s. str.* strain isolated from a disseminated cutaneous human sporotrichosis case presented a 10pb deletion in the ribosomal NTS (nontranscribed spacer) region when compared with the control strains obtained from fixed cutaneous sporotrichosis cases (Zhang et al. 2011). Nucleotide polymorphisms are also able to separate environmental and clinical *S. schenckii* strains. Two single-base transitions in the D1–D2 domain of rDNA differentiate strains from these groups (Criseo and Romeo 2010).

Genetic polymorphisms are also likely to be related to antifungal susceptibility in the *Sporothrix* complex. Through a haplotype network approach based on calmodulin and ITS sequences of 22 strains of *S. brasiliensis* and 39 strains of *S. schenckii s. str.*, it has been demonstrated that the epidemic species *S. brasiliensis* has a low genetic diversity and a small variability of susceptibilities to itraconazole and posaconazole. On the other hand, the *S. schenckii s. str.* strains were separated into ten haplotypes, which correlated with the high variability among minimal inhibitory concentrations to the drugs most commonly used in the sporotrichosis treatment (Rodrigues et al. 2014b).

Recently, Sasaki et al. (2014) reported the presence of intra and interspecies polymorphisms in chromosome number and size of 23 strains belonging to the *Sporothrix* complex. In this study, chromosomal polymorphisms and mapping of nine loci (β-tubulin, calmodulin, catalase, chitin synthase 1, ITS, Pho85 cyclin-dependent kinase, protein kinase C Ss-2, G protein a subunit, and topoisomerase II) were studied. The gene hybridization analysis showed that closely related species in

phylogenetic analysis had similar genetic organizations, mostly due to identification of synteny groups in chromosomal bands of similar sizes.

The *Sporothrix* complex is the last clinically relevant group of dimorphic fungi to have an elucidated genome sequence, thus limiting the molecular knowledge about the cryptic species of this complex. Recently, the genome sequence of the *S. schenckii* strain ATCC 58251 was described. The genome size was calculated as approximately 32 Mb with a GC content of 55 %. This genome comprises 8674 protein genes, 111 transfer RNA (tRNA) encoding genes, and 20 rRNA-associated genes (Cuomo et al. 2014). Despite the lack of whole genome information in the *Sporothrix* complex, some genes have been recently described, such as the α-subunit of the endoplasmic reticulum glucosidase II (Robledo-Ortiz et al. 2012), the α1,2-mannosyltransferase (Hernandez-Cervantes et al. 2012), a cytosolic phospholipase A2 (Valentin-Berrios et al. 2009), a guanosine diphosphatase (López-Esparza et al. 2013), an STE20-like protein (Zhang et al. 2013), a histidine kinase associated to dimorphism (Hou et al. 2013), and a calcium/calmodulin kinase gene (Valle-Aviles et al. 2007). These sequences are potential targets for the development of new identification and typing methods to be applied to the species of the *Sporothrix* complex.

2.5 Molecular Identification of *Sporothrix* Species

In recent years, the development of DNA-based methods to identify fungal isolates has led to a decrease in the time-consuming step of morphological identification, while maintaining or improving specificity, accuracy, and sensitivity. Until now, few molecular methods have been applied in the detection of *S. schenckii* DNA from clinical specimens and in the identification of *Sporothrix* spp. in culture (Table 2.1).

As described earlier in this chapter, the most reliable method for the identification of species in the *Sporothrix* complex is partial calmodulin gene sequencing

Table 2.1 Molecular studies for species identification in the *Sporothrix schenckii* complex

Method	Target gene region	Reference
PCR[a] sequencing	Calmodulin, β-tubulin, and chitin synthase genes	Marimon et al. (2007)
PCR sequencing	ITS[c], LSU[d], and β-tubulin	de Meyer et al. (2008)
PCR sequencing	ITS, β-tubulin, chitin synthase genes	Zhang et al. (2011)
PCR fingerprinting	T3B	Oliveira et al. (2012)
PCR sequencing	ITS	Zhou et al. (2014)
RFLP[b]	Calmodulin	Rodrigues et al. (2014c)

[a]Polymerase chain reaction
[b]Restriction Fragment Length Polymorphism
[c]Internal Transcriber Space
[d]Large subunit

(Marimon et al. 2007). After the description of *Sporothrix* complex, one important issue is the search for rapid methods of species identification and typing. Oliveira et al. (2012) reported a polymerase chain reaction (PCR) fingerprinting using the universal primer T3B to distinguish among species of the *Sporothrix* complex. T3B fingerprinting generated clearly distinct banding patterns, allowing the correct identification of all 35 clinical isolates at the species level, which was further confirmed by partial calmodulin gene sequence analyses. Overall, there was a 100 % agreement between the species identification using both genotypic methodologies. These profiles were also able to accurately distinguish the strains misidentified by phenotypic analysis. This proposed identification technique is simple, reliable, faster, less expensive, and requires less technical expertise than sequencing. The computer-scanned PCR profiles generated can form the basis of a computer database that can be used for future identification of atypical or unidentifiable *Sporothrix* isolates. This methodology is supposed to be an ideal routine identification system for clinical mycology laboratories, particularly in those with limited facilities or technical expertise.

In addition, a PCR-restriction fragment length polymorphism (RFLP) targeting the calmodulin gene sequence digested with the restriction enzyme *Hha*I was reported, with five different electrophoretic patterns representing the isolates of *Sporothrix* spp.: *S. brasiliensis*, *S. schenckii s. str.*, *S. globosa*, and *S. luriei*. However, this PCR-RFLP protocol did not permit identification of all species included in this complex, because *S. mexicana* and *S. pallida* yield identical band patterns (Rodrigues et al. 2014c).

In a search for other methodologies with enough discrimination power to differentiate strains of the *Sporothrix* complex, Zhou et al. (2014) suggested that the ITS region analysis could also be applied for identification at the species level. Another study applying the ITS1-5.8S-ITS2 region of the ribosomal DNA reported that this region could be utilized as a broad molecular marker for inter- and intraspecific genetic diversity of the *Histoplasma capsulatum* and *S. schenckii* species complexes and could discriminate *H. capsulatum* or *Sporothrix* isolates according to their geographic distribution and association with environmental sources. However, the authors reported that the ITS regions were able to distinguish neither *H. capsulatum* species nor *Sporothrix* spp. among their respective phylogenetic, biological, and/or taxonomic species complexes (Estrada-Bárcenas et al. 2014).

Only a few molecular tools exist for *Sporothrix* species identification and typing (Oliveira et al. 2012; Zhou et al. 2014; Rodrigues et al. 2014c), and the development of new typing methods are necessary. Further studies for the development of new methodologies for identification and typing of the *Sporothrix* complex should be easy since the genome sequence of *S. schenckii* (ATCC 58251) was recently reported (Cuomo et al. 2014), which will facilitate the study of this and of other species of the *Sporothrix* complex.

2.6 Conclusion and Perspectives

The species of the *Sporothrix* complex present several differences, both at molecular and at phenotypic levels. Rapid and accurate identification to the species level is crucial to correct management of sporotrichosis. Consequently, the correlation between molecular data and phenotypic characteristics is fundamental to the identification of the *Sporothrix* complex. Only a few molecular tools exist for *Sporothrix* species identification and typing. Therefore, better understanding of the strengths and weakness of currently available molecular methodologies would greatly improve the speciation and the intra-variability among the isolates.

References

Almeida-Paes R, Oliveira MM, Freitas DF et al (2014) Sporotrichosis in Rio de Janeiro, Brazil: *Sporothrix brasiliensis* is associated with atypical clinical presentations. PLoS Negl Trop Dis 8, e3094

Arrillaga-Moncrieff I, Capilla J, Mayayo E et al (2009) Different virulence levels of the species of *Sporothrix* in a murine model. Clin Microbiol Infect 15:651–655

Barros MBL, Almeida-Paes R, Schubach AO (2011) *Sporothrix schenckii* and sporotrichosis. Clin Microbiol Rev 24:633–654

Beer ZW, Harrington TC, Vismer HF et al (2003) Phylogeny of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 95:434–441

Berbee ML, Taylor JW (1992) 18S ribossomal RNA gene sequence characters place the human pathogen *Sporothrix schenckii* in the genus *Ophiostoma*. Exp Micol 16:87–91

Bubnick M, Smulian AG (2007) The MAT1 locus of *Histoplasma capsulatum* is responsive in a mating type-specific manner. Eukaryot Cell 6:616–621

Casselton LA (2008) Fungal sex genes-searching for the ancestors. Bioessays 30:711–714

Criseo G, Romeo O (2010) Ribosomal DNA sequencing and phylogenetic analysis of environmental *Sporothrix schenckii* strains: comparison with clinical isolates. Mycopathologia 169:351–358

Cuomo CA, Rodriguez-Del Valle N, Perez-Sanchez L et al (2014) Genome sequence of the pathogenic fungus *Sporothrix schenckii* (ATCC 58251). Genome Announc 2:e00446–14

de Meyer EM, de Beer ZW, Summerbell RC et al (2008) Taxonomy and phylogeny of new wood- and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 100:647–661

Dias NM, Oliveira MME, Santos C et al (2011) Sporotrichosis caused by *Sporothrix mexicana*, Portugal. Emerg Infect Dis 17:1975–1976

Dixon DM, Salkin IF, Duncan RA et al (1991) Isolation and characterization of *Sporothrix schenckii* from clinical and environmental sources associated with the largest U.S. epidemic of sporotrichosis. J Clin Microbiol 29:1106–1113

Estrada-Bárcenas DA, Vite-Garín T, Navarro-Barranco H et al (2014) Genetic diversity of *Histoplasma* and *Sporothrix* complexes based on sequences of their ITS1-5.8S-ITS2 regions from the BOLD System. Rev Iberoam Micol 31:90–94

Fernandes GF, Santos PO, Rodrigues AM et al (2013) Characterization of virulence profile, protein secretion and immunogenicity of different *Sporothrix schenckii* sensu stricto isolates compared with *S. globosa* and *S. brasiliensis* species. Virulence 4:241–249

Guarro J (2012) Taxonomía y biología de los hongos causantes de infección en humanos. Enferm Infecc Microbiol Clin 30:33–39

Gutierrez-Galhardo MC, Zancopé-Oliveira RM, Valle ACF et al (2008) Molecular epidemiology and antifungal susceptibility patterns of *Sporothrix schenckii* isolates from a cat-transmitted epidemic of sporotrichosis in Rio de Janeiro, Brazil. Med Mycol 46:141–151

Hernandez-Cervantes A, Mora-Montes HM, Alvarez-Vargas A et al (2012) Isolation of *Sporothrix schenckii* MNT1 and the biochemical and functional characterization of the encoded alpha1,2-mannosyltransferase activity. Microbiology 158:2419–2427

Hintz WE (1999) Sequence analysis of the chitin synthase A gene of the Dutch elm pathogen *Ophiostoma novo-ulmi* indicates a close association with the human pathogen *Sporothrix schenckii*. Gene 237:215–221

Hou B, Zhang Z, Zheng F et al (2013) Molecular cloning, characterization and differential expression of DRK1 in *Sporothrix schenckii*. Int J Mol Med 31:99–104

Kano R, Anzawa K, Mochizuki T et al (2013) *Sporothrix schenckii* (sensu strict *S. globosa*) mating type 1-2 (MAT1-2) gene. J Dermatol 40:726–730

Kano R, Tsui CK, Hamelin RC et al (2014) The MAT1-1:MAT1-2 Ratio of *Sporothrix globosa* Isolates in Japan. Mycopathologia. doi:10.1007/s11046-014-9808-7

Liu X, Lian C, Jin L et al (2003) Characterization of *Sporothrix schenckii* by random amplification of polymorphic DNA assay. Chin Med J 116:239–242

Liu TT, Zhang K, Zhou X (2014) Molecular identification of *Sporothrix* clinical isolates in China. J Zhejiang Univ Sci B 15:100–108

Lopes-Bezerra LM, Schubach AO, Costa RO (2006) *Sporothrix schenckii* and sporotrichosis. An Acad Bras Cienc 78:293–308

López-Esparza A, Álvarez-Vargas A, Mora-Montes HM et al (2013) Isolation of *Sporothrix schenckii* GDA1 and functional characterization of the encoded guanosine diphosphatase activity. Arch Microbiol 195:499–506

Madrid H, Cano J, Gene J et al (2009) *Sporothrix globosa*, a pathogenic fungus with widespread geographical distribution. Rev Iberoam Micol 26:218–222

Madrid H, Gene J, Cano J et al (2010) *Sporothrix brunneoviolacea* and *Sporothrix dimorphospora*, two new members of the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 102:1193–1203

Marimon R, Cano J, Gene J et al (2007) *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol 45:3198–3206

Marimon R, Gene J, Cano J, Guarro J (2008a) *Sporothrix luriei*: a rare fungus from clinical origin. Med Mycol 46:621–625

Marimon R, Serena C, Gene J et al (2008b) In vitro antifungal susceptibilities of five species of *Sporothrix*. Antimicrob Agents Chemother 52:732–734

Mesa-Arango AC, Reyes-Montes MR, Perez-Mejia A et al (2002) Phenotyping and genotyping of *Sporothrix schenckii* isolates according to geographic origin and clinical form of Sporotrichosis. J Clin Microbiol 40:3004–3011

Morris-Jones R (2002) Sporotrichosis. Clin Exp Dermatol 27:427–431

Morrison AS, Lockhart SR, Bromley JG et al (2013) An environmental *Sporothrix* as a cause of corneal ulcer. Med Mycol Case Rep 2:88–90

O'Reilly LC, Altman SA (2006) Macrorestriction analysis of clinical and environmental isolates of *Sporothrix schenckii*. J Clin Microbiol 44:2547–2552

Oliveira MME, Almeida-Paes R, Muniz MM et al (2011) Phenotypic and molecular identification of *Sporothrix* isolates from an epidemic area of sporotrichosis in Brazil. Mycopathologia 172:257–267

Oliveira MME, Sampaio P, Almeida-Paes R et al (2012) Rapid identification of *Sporothrix* species by T3B fingerprinting. J Clin Microbiol 50:2159–2162

Orofino-Costa R, Unterstell N, Gripp AC et al (2013) Pulmonary cavitation and skin lesions mimicking tuberculosis in a HIV negative patient caused by *Sporothrix brasiliensis*. Med Mycol Case Rep 2:65–71

Reis RS, Almeida-Paes R, Muniz MM et al (2009) Molecular characterisation of *Sporothrix schenckii* isolates from humans and cats involved in the sporotrichosis epidemic in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 104:769–774

Robledo-Ortiz CI, Flores-Carreon A, Hernandez-Cervantes A et al (2012) Isolation and functional characterization of *Sporothrix schenckii* ROT2, the encoding gene for the endoplasmic reticulum glucosidase II. Fungal Biol 116:910–918

Rodrigues AM, de Hoog GS, Camargo ZP (2013) Emergence of pathogenicity in the *Sporothrix schenckii* complex. Med Mycol 51:405–412

Rodrigues AM, de Hoog GS, Camargo ZP (2014a) Emerging sporotrichosis is driven by clonal and recombinant *Sporothrix* species. Emerg Microbes Infect 3, e32

Rodrigues AM, de Hoog GS, Pires DC et al (2014b) Genetic diversity and antifungal susceptibility profiles in causative agents of sporotrichosis. BMC Infect Dis 14:219

Rodrigues AM, de Hoog GS, Camargo ZP (2014c) Genotyping species of the *Sporothrix schenckii* complex by PCR-RFLP of calmodulin. Diagn Microbiol Infect Dis 78:383–387

Romeo O, Scordino F, Criseo G (2011) New insight into molecular phylogeny and epidemiology of *Sporothrix schenckii* species complex based on calmodulin-encoding gene analysis of Italian isolates. Mycopathologia 172:179–186

Sasaki AA, Fernandes GF, Rodrigues AM et al (2014) Chromosomal polymorphism in the *Sporothrix schenckii* complex. PLoS One 9, e86819

Suh DY, Hyun MW, Kim JJ et al (2013) *Ophiostoma ips* from Pinewood Nematode Vector, Japanese Pine Sawyer Beetle (*Monochamus alternatus*), in Korea. Mycobiology 41:59–62

Valentin-Berrios S, Gonzalez-Velazquez W, Perez-Sanchez L et al (2009) Cytosolic phospholipase A2: a member of the signalling pathway of a new G protein alpha subunit in *Sporothrix schenckii*. BMC Microbiol 9:100

Valle-Aviles L, Valentin-Berrios S, Gonzalez-Mendez RR et al (2007) Functional, genetic and bioinformatic characterization of a calcium/calmodulin kinase gene in *Sporothrix schenckii*. BMC Microbiol 7:107

Yu X, Wan Z, Zhang Z et al (2013) Phenotypic and molecular identification of *Sporothrix* isolates of clinical origin in Northeast China. Mycopathologia 176:67–74

Zhang Z, Liu X, Lv X et al (2011) Variation in genotype and higher virulence of a strain of *Sporothrix schenckii* causing disseminated cutaneous sporotrichosis. Mycopathologia 172:439–446

Zhang Z, Hou B, Zheng F et al (2013) Molecular cloning, characterization and differential expression of a *Sporothrix schenckii* STE20-like protein kinase *Ss*Ste20. Int J Mol Med 31:1343–1348

Zhou X, Rodrigues AM, Feng P et al (2014) Global ITS diversity in the *Sporothrix schenckii* complex. Fungal Divers 66:153–165

Chapter 3
Components and Virulence Factors of the *Sporothrix schenckii* Species Complex

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Abstract Sporotrichosis is a fungal infection caused by the *Sporothrix schenckii* species complex, which includes species of clinical relevance such as *S. brasiliensis*, *S. schenckii sensu stricto*, *S. globosa*, *S. mexicana*, and *S. luriei*. When *S. schenckii* was discovered as a species complex with several entities, the similarities and differences among these pathogenic species began to be studied with respect to their virulence, susceptibility to antifungal agents, protein production, and immunogenicity, among other characteristics. Still, little is known about the factors that contribute to the virulence of this species complex and about the mechanisms involved in the establishment and development of sporotrichosis. This chapter reviews the virulence factors and main components described for the *S. schenckii* complex, which include fungal dimorphism, thermotolerance, melanin production, secretion of proteases, and cell surface components.

Keywords *Sporothrix schenckii* complex • Virulence factors • Cell wall • Antigens

3.1 Introduction

Sporotrichosis is a subcutaneous mycosis that until this past century was attributed to only one species, *Sporothrix schenckii*. The frequency of this infection has increased in recent years, mainly in immunocompromised patients (López-Romero et al. 2011). Also in current years, the phylogeny and taxonomic status of this

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fungus have been widely studied through molecular biology methods, and it has been demonstrated that this fungus is indeed a complex of cryptic species (Marimon et al. 2006). Different *Sporothrix* species are now considered to be human pathogens: *S. brasiliensis*, *S. globosa*, *S. mexicana*, *S. luriei*, *S. pallida*, and *S. schenckii sensu stricto* (Marimon et al. 2007, 2008; de Meyer et al. 2008). All these species share the same ecological niche and very similar phenotypic traits, and they all are dimorphic fungi; that is, they exhibit a mycelial morphotype in nature and a parasitic yeast morphotype in the host. All the information regarding its biological characteristics has been studied for one species, *S. schenckii*, and it has only been in this recent century that other species have been identified and that work to clarify specific characteristics has just begun.

The "damage-response framework" of microbial pathogenesis of Casadevall and Pirofski (1999, 2001, 2003) refers to the interaction among microbes, host, and environment. The damage to the host may be given by either the microbial factors (or virulence factors) or the host response. To date, information regarding the microbial pathogenesis in the establishment and development of sporotrichosis and specific virulence factors of *Sporothrix* spp. is still scarce. Current data on the *Sporothrix* species complex virulence in animal models has shown *S. brasiliensis* to be the most virulent species, followed by *S. schenckii s. str.*, *S. globosa*, and *S. mexicana* (Arillaga-Moncrieff et al. 2009).

In this chapter, microbial factors (virulence factors), such as dimorphism, thermotolerance, melanin production, proteases, and cell wall (CW) biochemical components are discussed, mainly in connection with *S. schenckii*, although all available information concerning other relevant clinical species is also included. In Chaps. 4 and 7, these and other virulence factors of *S. schenckii* are discussed again, highlighting their role in the environment and during the infectious process.

3.2 Dimorphism

Dimorphism is the ability of some fungal agents to exhibit a phenotypic duality coupled with a cellular differentiation process, which in turn may be related to pathogenicity and virulence mechanisms. In sporotrichosis, as well as in other subcutaneous and systemic mycoses, fungi exhibit an infectious mycelial morphotype distributed in their specific ecological niche that switches to a parasitic yeast morphotype when introduced in their host. The mycelial and yeast morphotypes can also be easily obtained at 25 °C and 35–37 °C, respectively, with appropriate culture media in laboratory conditions. Conidia of the mycelial morphotype vary according to each species (Marimon et al. 2007), but, in general, mycelia show hyaline, septate, thin hyphae, with sympodially borne conidia, singly or in groups, whereas sessile conidia, in some cases, with time of incubation, develop thick walls and a dark brown pigment, generally along the sides of hyphae (Fig. 3.1a, b).

Mycelial morphotype colonies, initially described as white and glabrous, in time become wrinkled and membranous with black areas (Fig. 3.2a, b). Yeast

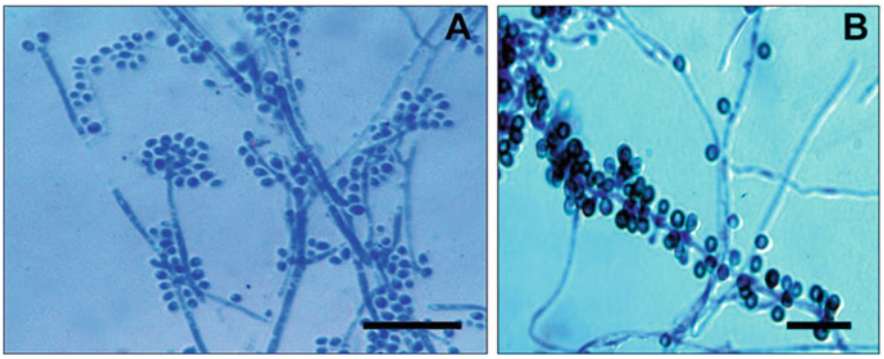

Fig. 3.1 *Sporothrix schenckii* EH-143 mycelial morphotype. (**a**) Hyaline, septate, thin hyphae with sympodially borne conidia. (**b**) Thick-walled, dark brown sessile conidia along the sides of hyphae. Bars = 10 μm

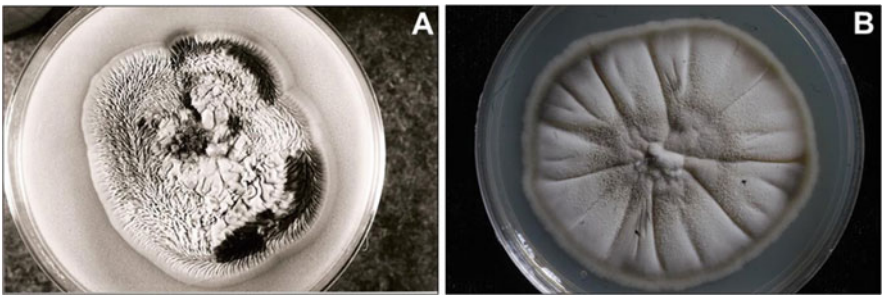

Fig. 3.2 Mycelial morphotype colonies. (**a**) *Sporothrix schenckii* EH-143 and (**b**) *Sporothrix globosa* EH-230, at 28 °C on potato dextrose agar medium for 15 days

morphotype cells are fusiform and ovoid with single, double, or multiple budding; although scarcely observed in human biological samples (asteroid bodies), they are generally described and easily observed as "cigar bodies" in experimental host tissues (Fig. 3.3).

The dimorphic transition of *S. schenckii* was first described by Howard in 1961; since then, environmental changes such as moisture, pH, temperature, nutrients, oxygen availability, G proteins, and calcium uptake, among others, have been mentioned as factors with an effect on this transition. In response to these changes, the fungus modifies its physiology and morphology to be able to cope with new physiological conditions and to evade the immune response of the host (Szaniszlo 1985). This dimorphism, both in its ecological niches and within different hosts (Casadevall et al. 2003), is not essential for its life cycle, but it is relevant for its pathogenicity (Nemecek et al. 2006; Gauthier and Klein 2008).

Dimorphism in *S. schenckii* has rarely been studied when compared with other human pathogenic dimorphic fungi; however, since 1983, studies by Rodríguez del Valle et al. (1983) have demonstrated that this fungus dimorphic switch is controlled not only by temperature but also by the pH of the culture medium, relevant

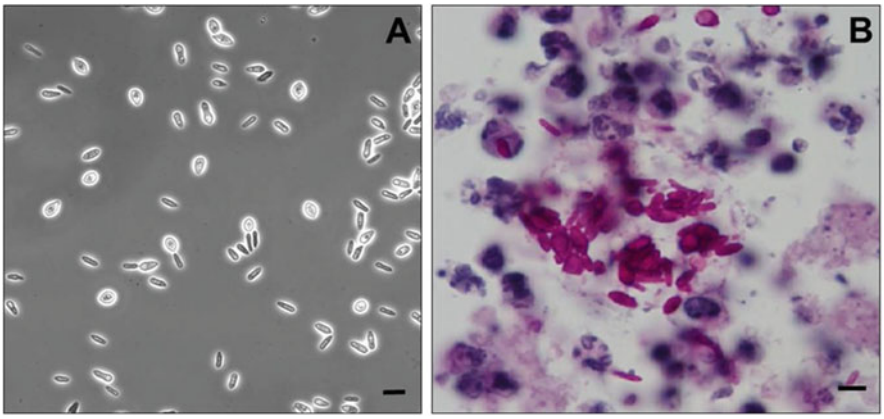

Fig. 3.3 *Sporothrix schenckii* EH-143 yeast morphotype. (**a**) Yeast budding cells at 37 °C on YPG (yeast extract-peptone-glucose) medium. (**b**) Single yeast cells (cigar bodies) within macrophages and neutrophils but abundant PAS-positive yeast cells are observed inside a foam cell in *S. schenckii* EH-143-infected mice at 8 weeks of infection. Bars = 10 μm

in the development of a particular morphotype. Mycelial morphotype without yeast cells was obtained at 25 °C and low pH (4.0–5.0); neutral to alkaline pH favored the yeast morphotype. Aeration of cultures favored the yeast morphotype in a pH range from 6.0 to 8.0 at 25 °C. Since these investigations concerning the *S. schenckii* dimorphic switch were conducted, other works have been carried out with reference to calcium uptake (Serrano and Rodriguez del Valle 1990; Rivera-Rodríguez and Rodríguez del Valle 1992), and a calcium/calmodulin-dependent protein kinase (CaMK), encoded by the calcium/calmodulin kinase I (*sscmk1*) gene, has been described (Valle-Avilés et al. 2007). Using CaMK inhibitors, these authors demonstrated that the transition from yeast cells to hyphae was inhibited, thereby suggesting a calcium/calmodulin pathway in the regulation of *S. schenckii* dimorphism. The same phenomenon was demonstrated by RNA interference (RNAi) technology, with RNAi transformants being unable to grow as yeast cells at 35 °C (Rodríguez-Caban et al. 2011).

Protein kinase C (PKC) is an important signal transduction enzyme and a family of proteins made up of different isoforms described in different fungi. Studies concerning the response to PKC effector molecules during the induction of the yeast-to-mycelium transition indicated the presence and involvement of this enzyme in *S. schenckii* dimorphism (Colon-Colón and Rodríguez del Valle 1993). In later studies, two PKC-like genes were identified in *S. schenckii* and, using reverse transcription-polymerase chain reaction (RT-PCR), the *pkcSs-2* gene was found to be expressed at all intervals tested during the yeast-to-mycelium transition in this fungus (Aquino-Piñero & Rodríguez del Valle 2002).

In other dimorphic human fungal pathogens as *Blastomyces dermatitidis* and *Histoplasma capsulatum*, a long-sought regulator that controls the switch from the mycelial morphotype to a pathogenic yeast morphotype was found by Nemecek

et al. (2006). This control corresponded to DRK1, a hybrid dimorphism-regulating histidine kinase that regulates dimorphism and virulence for both of the above-mentioned fungi. DRK1 is required for the transition from the mycelial to the pathogenic yeast morphotype, the expression of virulence genes, and pathogenicity in vivo. Recently, Hou et al. (2013), by means of molecular cloning, characterization, and differential expression, obtained the partial complementary DNA (cDNA) sequence of DRK1 of *S. schenckii*, designated *SsDRK1*. Quantitative real-time RT-PCR revealed that *SsDRK1* was more highly expressed in the yeast stage than in the mycelial stage, indicating that it may be involved in the dimorphic transition of *S. schenckii*.

Other research projects are in process concerning additional proteins (Valentin-Berrios et al. 2009; González-Velazquez et al. 2012; Zhang et al. 2012, 2013) and lipids (Kitajima 2000) in the dimorphic transition of *S. schenckii*. Information regarding the dimorphic switch of *Sporothrix* spp., which is now essential to help us understand the mechanism of pathogenesis of this enigmatic *S. schenckii* species complex, is only starting to flow.

3.3 Thermotolerance

Not all isolates of *S. schenckii* from the environment have the ability to adapt to the temperature of the host's body. In 1979, Kwon-Chung showed that cutaneous strains of clinical origin are able to grow in vitro at 35 °C but not at 37 °C. Previous studies with young male mice inoculated intraperitoneally and intracardially showed that the lesions were more pronounced when mice were kept at lower room temperatures, thereby suggesting a connection between thermotolerance and virulence (Mackinnon and Conti-Díaz 1962; Hogan et al. 1996). De Albornoz et al. (1986) found that different isolates, in fixed cutaneous or disseminated form, can grow either at 27 °C or at 35 °C. In some recent work, isolates from Colombia with low thermotolerance showed a higher incidence of fixed cutaneous sporotrichosis; in contrast, isolates from Mexico with a higher thermotolerance showed a higher incidence of lymphocutaneous sporotrichosis (Mesa-Arango et al. 2002). Clinically, local thermotherapy has an excellent therapeutic effect and has been found to increase the rate of death of neutrophils in short-term assays (Hogan et al. 1996). The factor or genes responsible for thermotolerance are still unknown, as are the differences exhibited by the diverse clinically relevant species of *Sporothrix*.

3.4 Melanin Production

Melanin is considered a large group of polymers with diverse molecular structures typically with a black or dark brown color, formed by the oxidative polymerization of phenolic or indolic compounds. In fungi, melanin is synthesized in the cytoplasm and deposited in the CW or excreted as an extracellular polymer (Eisenman and Casadevall 2012). These pigments are not essential for fungal growth and development (Hogan et al. 1996). There are two types of melanin among fungi. The most frequent ones are 1,8-dihydroxynaphthalene melanin (DHN-melanin) as in *Aspergillus fumigatus* (Latgé 2001) and melanin via dihydroxyphenylalanine (DOPA) by which tyrosinases or laccases hydroxylate DOPA to dopaquinone (Langfelder et al. 2003) as in *Cryptococcus neoformans* (Polacheck and Kwon-Chung 1988). Other fungi produce still another type of soluble melanoid pigment from L-tyrosine called pyomelanin (Almeida-Paes et al. 2012). Observations suggest that melanin contributes to the virulence of fungal agents by protecting them from the host defense response, oxidizing agents, and hydrolytic enzymes, and reducing phagocytosis or the induction of cell death (Jacobson 2000). The *S. schenckii* species complex is able to synthesize melanin, which is evident in the varied colony pigmentation shown by different strains (Fig. 3.2). Generally, a fungal colony isolated from the patient initially shows a creamish color; the pigmentation then increases during the incubation time until, finally, some colonies turn black after 3 or 4 weeks (Rippon 1988). The presence of melanin has been associated with the virulence of this fungus (Rippon 1988; Hogan et al. 1996; Almeida-Paes et al. 2009; Madrid et al. 2010). *S. schenckii* has the ability to produce melanin in a very wide pH range, which is considered a survival advantage (Almeida-Paes et al. 2009). Melanin production varies in different strains of the fungus, and it has been reported that the strains with more melanin cause a faster infection than those that produce less pigment (Almeida-Paes et al. 2009; Madrid et al. 2010). These findings suggest that the melanized conidia CW prevents *S. schenckii* from being killed, enhances protection from ultraviolet (UV) solar irradiation, and, during infection, it affects host defense mechanisms by reducing phagocytosis and scavenging reactive oxygen and nitrogen species. Furthermore, melanized conidia from a *S. schenckii* wild type were more resistant than conidia from two melanin-deficient mutants to oxidant killing in vitro and to phagocytosis by human monocytes and murine macrophages (Romero-Martinez et al. 2000).

There is now evidence that *S. schenckii* isolates have the capacity to produce DHN- and DOPA-melanin (Romero-Martinez et al. 2000; Almeida-Paes et al. 2009, 2012), with this last pigment accumulating on the fungal CW of conidia, yeast cells, and hyphae (Almeida-Paes et al. 2009). Furthermore, recent research showed that not only *S. schenckii* but also *S. brasiliensis* and *S. globosa* were able to produce pyomelanin in the presence of tyrosine, thereby suggesting that this pigment could be involved in virulence (Almeida-Paes et al. 2012).

It appears that future research on microbial factors will have to allow for the clarification of so many unanswered questions regarding *Sporothrix* virulence.

3.5 Proteases

It has been postulated that certain extracellular enzymes, such as acid phosphatase, play an important role in the interaction of the yeast forms of *S. schenckii* with macrophages and other host cells (Garrison and Arnold 1983; Hogan et al. 1996). The activity of acid phosphatase is produced by conidia, mycelia, and the yeast forms of *S. schenckii*; greater amounts of activity are associated with the yeast extracts (Arnold et al. 1986; Hogan et al. 1996). Other studies have reported two different extracellular proteinases when *S. schenckii* is grown in media containing albumin and collagen as a nitrogen source (Tsuboi et al. 1987). Proteinase I is a 36.5 kDa serine protease that is inhibited by chymostatin, with an optimum pH of 6.0. Proteinase II is a 39 kDa aspartyl proteinase with an optimum pH of 3.5. The ability of *S. schenckii* to invade cutaneous tissues is associated with proteinases. Experimental mouse infections appear to confirm this, since the inoculation of inhibitors of these enzymes largely suppresses the formation of nodules and promotes spontaneous healing (Hogan et al. 1996). Recent studies by Sandoval-Bernal et al. (2010) suggest that the damage to the epithelial monolayer caused by the interaction of *S. schenckii* yeasts may be mediated by the action of proteases such as serine and aspartyl proteases, acid phosphatase, collagenase, and gelatinase, all of which have been thought to have a key role in the pathogenicity of the *S. schenckii* complex (Lima and Lopes Bezerra 1997). The presence of proteases can vary depending on the virulence of the species of the *S. schenckii* complex. Fernandes et al. (2013) found that highly virulent strains from isolates of *S. schenckii* show a number of secreted enzymes, such as proteinases, caseinases, gelatinases, DNase, and ureases, which have not been observed in the hypervirulent species of *S. brasiliensis*.

3.6 Cell Surface Components

The virulence of the *S. schenckii* complex has also been attributed to the presence of certain cell components involved in the interactions with the host, such as adhesins present on the cell surface, which increase adhesion to epithelial cells and extracellular matrix (Lima et al. 1999; Teixeira-Castelo et al. 2009; Ruiz-Baca et al. 2009), or to the ability to revert ergosterol peroxide to ergosterol, which has been proposed as an evasion mechanism of the fungal response (Sgarbi et al. 1997).

The CW of *S. schenckii* consists of alkali-soluble and alkali-insoluble glucans found in the same proportion in the two morphological phases of the fungus (Previato et al. 1979; López-Romero et al. 2011). The cell surface polysaccharides appear to influence macrophage function. In vitro studies have shown that yeast phagocytosis by peritoneal macrophages is inhibited by purified galactomannans and rhamnomannans from the cell surface of *S. schenckii* (Oda et al. 1983). One of its main components is a peptidorhamnomannan (Fig. 3.4), which was isolated from

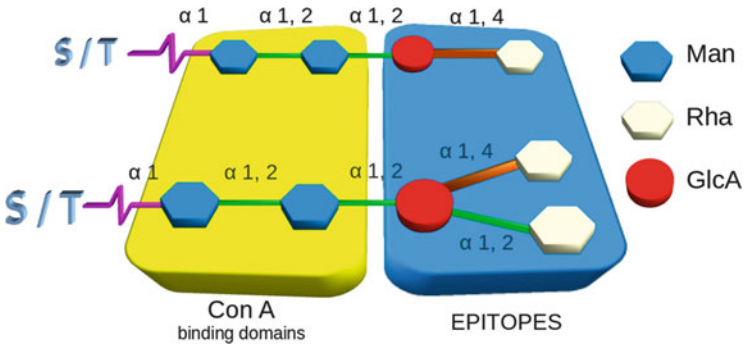

Fig. 3.4 General structure of the main antigenic epitopes and Con-A binding sites, described on the peptidorhamnomannan, both morphotypes (yeast and mycelium) of *S. schenckii* (Modified: Lopes-Alves et al. 1992)

the CW of *S. schenckii* yeast morphotype, a compound of polysaccharides such as D-mannose (50 %), L-rhamnose (33 %), galactose (1 %), and a peptide fraction of about 16 % (Lloyd and Bitoon 1971; Travassos et al. 1977; Lopes-Bezerra 2011; López-Romero et al. 2011).

The peptidorhamnomannans reacted with the carbohydrate-binding protein concanavalin A (ConA); this reactivity was associated with the presence of *O*-glycosidally linked chains. The peptidorhamnomannans are also recognized by antisera from patients with sporotrichosis, and it is thus capable of stimulating the immune mechanisms of these patients (Lloyd and Bitoon 1971; Travassos et al. 1977). The major immunogens of peptidorhamnomannans are associated with its carbohydrate residues, which are linked by *N*-glycosidic and *O*-glycosidic bonds present in both fungal morphotypes (Lopes-Alves et al. 1992, 1994; Lopes-Bezerra 2011). Fernandes et al. (1999) suggest that the *S. schenckii* conidia virulence may be determined by the CW sugar composition, with a molar ratio of rhamnose:mannose of 1.7:1.0 in cells grown for 4 days, and a ratio of 1.0:1.7 in conidia grown for 12 days.

Another component of the CW of *S. schenckii*, but of a lipid nature, seems to play an important role in the development of fungal mycosis, since it was able to inhibit it in in vitro phagocytosis assays (Carlos et al. 2003). The polysaccharide peptide, or peptidorhamnomannan, and the lipid antigen have been extensively studied: the kind of response they induce (Carlos et al. 1999, 2003; Maia et al. 2006; Verdan et al. 2012; Alegranci et al. 2013), the receptors involved in their recognition (Sassá et al. 2009, 2012; Negrini et al. 2013, 2014), and the corresponding signaling pathways that are activated (Gonçalves et al. 2015). However, which molecules are responsible for each of these effects is still not well known because they are handled as extracts not as a pure antigen.

One of the main antigens detected in the CW of both morphologies of *S. schenckii* is an adhesin-like glycoprotein with an apparent molecular weight of 70 kDa (gp70) (Fig. 3.5) that mediates the interaction of the fungus with

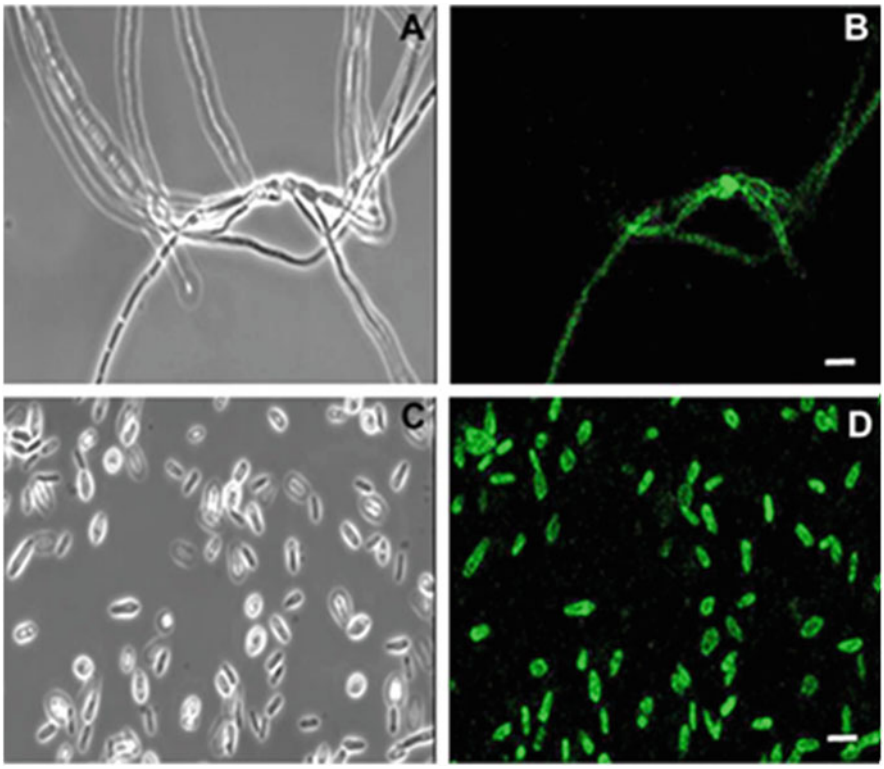

Fig. 3.5 Immunolocalization of gp70 in *S. schenckii* ATCC 58251 by confocal microscopy. Mycelium (**a**, **b**) and yeast cells (**c**, **d**). Bars = 10 μm

extracellular matrix proteins and the host tissue (Ruiz-Baca et al. 2009; Teixeira-Castelo et al. 2009). Passive immunized mice with monoclonal antibodies against gp70 showed a *S. schenckii* infection that was less severe, but inflammatory response seems to be promoted, according to the cytokine profile expressed (Nascimento et al. 2008). gp70 is distributed in different subcellular compartments and is not restricted to the CW of *S. schenckii* and *S. brasiliensis* yeast-like cells, according to transmission electron microscopy studies. Furthermore, gp70 was detected in the extracellular space, suggesting that it could also be secreted (Castro et al. 2013), and has been associated with the virulence of *S. schenckii* complex species that kill infected mice (Fernandes et al. 2013). However, in more recent studies, an inverse relationship was found between the expression of this antigen and the virulence of clinical isolates of *S. brasiliensis* (Castro et al. 2013). gp70 was characterized by mass spectrometry, and the peptide sequences identified in the genome of *S. schenckii* and *S. brasiliensis* corresponded to a 3-carboxymuconate cyclase, an enzyme involved in the degradation of benzoate (Castro et al. 2013). Studies of the CW of *S. schenckii s. str.*, *S. brasiliensis*, and *S. globosa* using two-dimensional polyacrylamide gels (2D PAGE) and Western blot (Fig. 3.6)

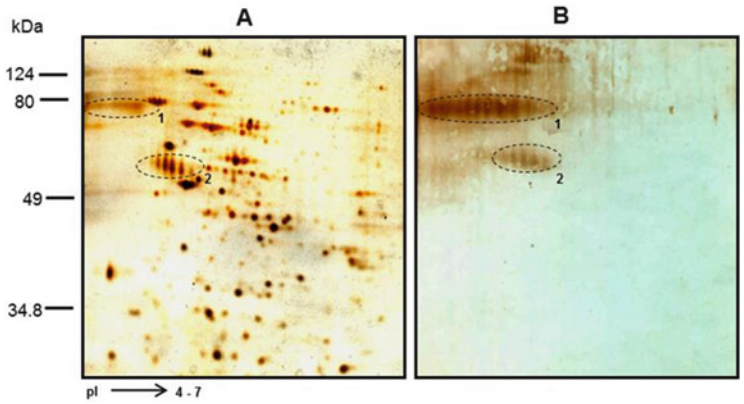

Fig. 3.6 2D-gel electrophoresis and immunoblotting analysis of CW proteins from *S. schenckii*. Proteins extracted from the CW of the yeast morphotype of *S. schenckii* ATCC 58251 were analyzed by 2D PAGE in gels that were stained by silver (**a**) or immunoblotted (**b**). Proteins marked with broken lines as 1 and 2 correspond to antigens gp70 (p*I*s 4.0–5.0) and gp60 (p*I*s 4.5–5.5), respectively (Ruiz-Baca et al. 2011)

identified two immunoreactive antigens of glycoprotein nature (Fig. 3.7) with apparent molecular weights of 70 (gp70) and 60 (gp60) kDa (Ruiz-Baca et al. 2011; Ruiz-Baca et al. 2014). However, characterization of peptide sequences by mass spectrometry indicated that gp70 and gp60 antigens corresponded to a 3-carboxymuconate cyclase.

A recent study evaluates the protein secretion of *S. brasiliensis*, *S. globosa*, and *S. schenckii s. str.* to define a virulence profile and connects it with the humoral immune response induced by these species (Fernandes et al. 2013). A great deal of heterogeneity of virulence among the different isolates of *S. schenckii s. str.* species was observed, and no correlation was found between virulence profile of isolates with thermotolerance or geographical origin. The most virulent strain induced mortality in a short time of infection, with a high fungal load mainly in lungs and spleen, colonizing the evaluated organs. This contrasted with a non-virulent strain, because no fungus was recovered from any organ, and infected mice survived to the end of the experiment. Interestingly, the most virulent isolate (*S. schenckii s. str.*) expressed less virulence factors as proteinase, caseinase, gelatinase, urease, and DNase activities, showing that the mechanism of pathogenesis is much more complex, involving these virulence factors and other molecules for evasion of the immune system (Fernandes et al. 2013). All of the isolates, including *S. brasiliensis* and *S. globosa*, secreted 60- and 46-kDa molecules, and probably represent important components that are common to all of the studied species. All isolates that had the 60-kDa molecule recognized by antisera from infected mice could kill them. On the other hand, sera from mice infected with non-virulent isolates did not recognize the 60-kDa molecule, which could be the immunodominant molecule in the *S. schenckii* complex. Using an immunoproteomic approach, Rodrigues et al. (2015) characterized proteins of potential significance in pathogenesis and

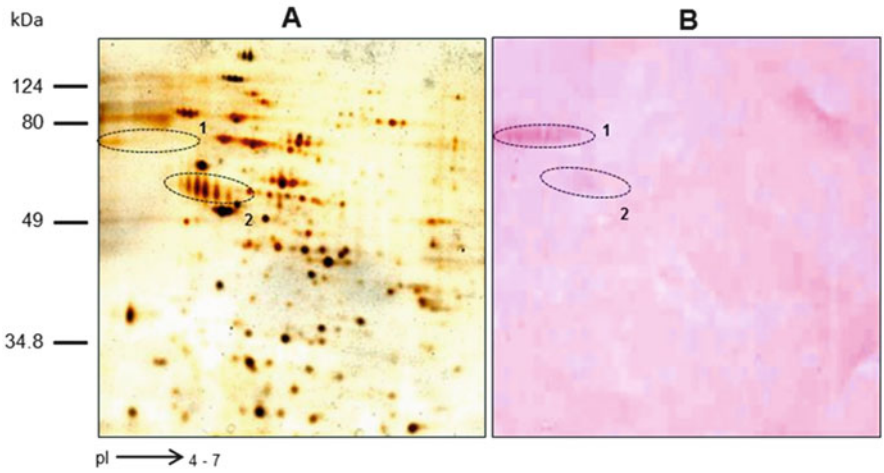

Fig. 3.7 Glycoprotein staining of 2D-PAGE of *S. schenckii* ATCC 58251 CW protein. Proteins extracted with hot SDS from yeast-like cells were analyzed by 2D-PAGE in gels that were stained by silver (**a**) or transfer to nitrocellulose membranes (**b**), they were stained with periodic acid-Schiff (PAS) reagent. Stained glycoproteins are indicated by spot numbers: 1, 70 kDa (p*I*s 4.0–5.0); 2, 60 kDa (p*I* 4.5–5.5) (Ruiz-Baca et al. 2011)

invasion that trigger the humoral response during human sporotrichosis. The data showed gp70 to be a cross-immunogenic protein shared among pathogenic *Sporothrix* spp. (*S. brasiliensis*, *S. schenckii*, and *S. globosa*) but absent in the ancestral environmental (non-virulent) *S. mexicana*, supporting the hypothesis that gp70 plays key roles in pathogenicity. Also, identified by MALDI-TOF (MS/MS), the major antigen of human sporotrichosis (gp60–70) is 3-carboxymuconate cyclase. According to Rodrigues et al. (2015), a convergent humoral response highlights 3-carboxymuconate cyclase as an important target for serological diagnosis and for vaccine development among phylogenetically related agents of sporotrichosis. The MS/MS data showed that the 60 kDa molecule reported as a virulence factor in the *S. schenckii* complex by Fernandes et al. (2013) is a variant of gp70, as both gp60 and gp70 were identified with the same peptide sequences. gp70/gp60 is a highly polymorphic protein, experimentally ranging from 55 to 73 kDa and 4.33 to 4.85 p*I*. These physicochemical variations seem to be the result of post-translational modifications that include isoforms and/or glycoforms. Furthermore, the protein regarded as 60 kDa is a complex of iso-glycoforms oscillating between 60 and 70 kDa. Other immunoreactive proteins in human sporotrichosis, related to signal transduction, pathogenicity, or metabolic/energetic processes, such as F-type H+-transporting ATPase subunit beta, saccharopepsin, signal peptidase protein, guanine nucleotide-binding protein (G protein), and catalase/peroxidase, have been identified. In addition, a common hypothetical protein was identified in *S. brasiliensis*, *S. schenckii*, and *S. globosa*, and an exclusive hypothetical protein in *S. mexicana*.

More studies should be conducted to characterize virulence profiles and virulence factors, correlating the pigmentation of conidia (Romero-Martinez et al. 2000), thermotolerance (Dixon et al. 1991), routes of infection (Tachibana et al. 1998), origin (Kong et al. 2006; Brito et al. 2007), culture conditions and CW (Fernandes et al. 1999), and secreted components (Fernandes et al. 2013), to the immunogenicity among the *S. schenckii* complex for a better understanding of sporotrichosis pathogenesis.

3.7 Conclusions and Future Perspectives

Although some advances have been made in the identification of components and factors of virulence in the *S. schenckii* complex, much work remains to be done. Some of the biggest problems we face when dealing with this dimorphic fungus are its genetic complexity and the lack of an adequate transformation system to characterize virulence factors. The recent release of the genome of *S. schenckii* and of *S. brasiliensis* will promote molecular studies and comparative genomics, as well as transcriptomic and proteomic analyses, of the components and virulence factors involved in the pathogenesis of the *S. schenckii* species complex. This will contribute to the discovery and characterization of new molecules with therapeutic target potential, and of vaccines against this mycosis of global importance.

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References

Alegranci P, Abreu-Ribeiro LC, Souza-Ferreira L et al (2013) The predominance of alternatively activated macrophages following challenge with cell wall peptide-polisaccharide after prior infection with *Sporothrix schenckii*. Mycopathologia 176:57–65

Almeida-Paes R, Frases S, Fialho MPC et al (2009) Growth conditions influence melanization of Brazilian clinical *Sporothrix schenckii* isolates. Microbes Infect 11:554–562

Almeida-Paes R, Frases S, Araújo Gde S et al (2012) Biosynthesis and functions of a melanoid pigment produced by species of the *Sporothrix* complex in the presence of L-tyrosine. Appl Environ Microbiol 78:8623–8630

Aquino-Piñero E, Rodriguez del Valle N (2002) Characterization of a protein kinase C gene in *Sporothrix schenckii* and its expression during the yeast-to-mycelium transition. Med Mycol 40:185–199

Arillaga-Moncrieff CJ, Mayayo E et al (2009) Different virulence levels of the species of *Sporothrix* in a murine model. Clin Microbiol Infect 15:651–655

Arnold WN, Mann LC, Sakai KH et al (1986) Acid phosphatases of *Sporothrix schenckii*. J Gen Microbiol 132:3421–3432

Brito MM, Conceição-Silva F, Morgado FN et al (2007) Comparison of virulence of different *Sporothrix schenckii* clinical isolates using experimental murine model. Med Mycol 45: 721–729

Carlos IZ, Sgarbi GBD, Placeres CP (1999) Host organism defense by a peptide-polysaccharide extracted from the fungus *Sporothrix schenckii*. Mycopathologia 144:9–14

Carlos IZ, Sgarbi DBG, Santos GC et al (2003) *Sporothrix schenckii* inhibits macrophage phagocytosis: Involvement of nitric oxide and tumour necrosis factor-α. Scand J Immunol 57:214–220

Casadevall A, Pirofski LA (1999) Host-pathogen interactions: Redefining the basic concepts of virulence and pathogenicity. Infect Immun 67:3703–3713

Casadevall A, Pirofski LA (2001) Host-pathogen interactions: the attributes of virulence. J Infect Dis 184:337–344

Casadevall A, Pirofski LA (2003) The damage-response framework of microbial pathogenesis. Nat Rev Microbiol 1:17–24

Casadevall A, Steenbergen JN, Nosanchuk JD (2003) Ready made virulence and dual use virulence factors in pathogenic environmental fungi the *Cryptococcus neoformans* paradigm. Curr Opin Microbiol 6:332–337

Castro RA, Kubitschenk-Barreira PH, Teixeira PA et al (2013) Differences in cell morphometry, cell wall topography and gp70 expression correlate with the virulence of *Sporothrix brasiliensis* clinical isolates. PLoS One 8, e75656

Colón-Colón W, Rodríguez del Valle N (1993) Studies on phase transitions in *Sporothix schenckii*: possible involvement of protein kinase C. In: Vanden Bossche H, Odds FC, Kerridge D (eds) Dimorphic fungi in biology and medicina. Plenum, New York, pp 225–246

de Albornoz MB, Mendoza M, de Torres ED (1986) Growth temperatures of isolates of *Sporothrix schenckii* from disseminated and fixed cutaneous lesions of sporotrichosis. Mycopathologia 95: 81–83

de Meyer EM, de Beer W, Summerbell RC et al (2008) Taxonomy and phylogeny of new wood- and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. Mycologia 100:647–661

Dixon DM, Salkin IF, Duncan RA et al (1991) Isolation and characterization of *Sporothrix schenckii* from clinical and environmental sources associated with the largest U.S. epidemic of sporotrichosis. J Clin Microbiol 29:1106–1113

Eisenman HC, Casadevall A (2012) Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol 93:931–940

Fernandes GS, Oliveira-dos Santos P, Rodrigues AM et al (2013) Characterization of virulence profile, protein secretion and immunogenicity of different *Sporothrix schecnkiisensu stricto* isolates compared with *S. globosa* and *S. brasiliensis*. Virulence 4:241–249

Fernandes KS, Mathews HL, Lopes Bezerra LM (1999) Differences in virulence of *Sporothrix schenckii* conidia related to culture conditions and cell-wall components. J Med Microbiol 48: 195–203

Garrison RG, Arnold WN (1983) Cytochemical localization of acid phosphatases in the dimorphic fungus *Sporothrix schenckii*. Curr Microbiol 9:253–258

Gauthier G, Klein BS (2008) Insights into fungal morphogenesis and immune evasion: fungal conidia, when situated in mammalian lungs, may switch from mold to pathogenic yeasts or spore-forming spherules. Microbe Wash DC 3:416–423

Gonçalves AC, Maia DCG, Ferreira LS et al (2015) Involvement of major components from *Sporothrix schenckii* cell wall in the caspase-1 activation, nitric oxide and cytokines production during experimental sporotrichosis. Mycopathologia 179:21–30

González-Velazquez W, Gonzalez-Mendes R, Rodriguez del Valle N (2012) Characterization and ligand identification of a membrane progesterone receptor in fungi: existence of a novel PAQR in *Sporothix schenckii*. BMC Microbiol 12:194

Hogan LH, Bruce SK, Levitz SM (1996) Virulence factors of medically importan fungi. Clin Microbiol Rev 9:469–488

Hou B, Zhang Z, Zheng F et al (2013) Molecular cloning, characterization and differential expression of DRK1 in *Sporothrix schenckii*. Int J Mol Med 31:99–104

Howard DH (1961) Dimorphism of *Sporotrichum schenckii*. J Bacteriol 81:464–469

Jacobson ES (2000) Pathogenic roles for fungal melanins. Clin Microbiol Rev 13:708–717

Kitajima Y (2000) Structural and biochemical characteristics of pathogenic fungus: cell walls, lipids and dimorphism, and action modes of antifungal agents. Nippon Ishinkin Gakkai Zasshi 41:211–217

Kong X, Xiao T, Lin J et al (2006) Relationships among genotypes, virulence and clinical forms of *Sporothrix schenckii* infection. Clin Microbiol Infect 12:1077–1081

Kwon-Chung KJ (1979) Comparison of isolates of *Sporothrix schenckii* obtained from fixed cutaneous lesions with isolates from other types oflesioins. J Infect Dis 139:424–431

Langfelder K, Streibel M, Jahn B et al (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 38:143–158

Latgé JP (2001) The pathobiology of *Aspergillus fumigatus*. Trends Microbiol 9:382–389

Lima OC, Lopes Bezerra LM (1997) Identification of Concanavalin A-binding antigen of the cell surface of *Sporothrix schenckii*. J Med Vet Mycol 35:167–172

Lima OC, Figueiredo CC, Pereira BA et al (1999) Adhesion of the human pathogen *Sporothrix schenckii* to several extracellular matrix proteins. Braz J Med Biol Res 32:651–657

Lloyd KO, Bitoon MA (1971) Isolation and purification of a peptidorhamnomannan from the yeast form of *Sporothrix schenckii* structural and immunochemical studies. J Immunol 107:663–671

Lopes-Alves LM, Mendoca-Previato L, Fournet B et al (1992) *O*-glycosidically linked oligosaccharides from peptidorhamnomannans of *Sporothrix schenkii*. Glycoconj J 9:75–81

Lopes-Alves L, Travassos LR, Previato JO et al (1994) Novel antigenic determinants from peptidorhamnomannans of *Sporothrix schenckii*. Glycobiology 4:281–288

Lopes-Bezerra LM (2011) *Sporothrix schenckii* cell wall peptidorhamnomannans. Front Microbiol 2:243

López-Romero E, Reyes-Montes MR, Pérez A et al (2011) *Sporothrix schenckii* complex and sporotrichosis, an emerging health problem. Future Microbiol 6:85–102

Mackinnon JE, Conti-Díaz IA (1962) The effect of temperature on sporotrichosis. Sabouraudia 2: 56–59

Madrid IM, Xavier MO, Mattei AS et al (2010) Role of melanin in the pathogenesis of cutaneous sporotrichosis. Microbes Infect 12:162–165

Maia DC, Sassá MF, Polesi MC (2006) Influence of Th1/Th2 cytokines and nitric oxide in murine systemic infection induced by *Sporothrix schenckii*. Mycopathologia 161:11–19

Marimon R, Gene J, Cano J et al (2006) Molecular phylogeny of *Sporothrix schenckii*. J Clin Microbiol 44:3251–3256

Marimon R, Cano J, Gene J et al (2007) *Sporothrix brasiliensis, Sporothrix globosa* and *Sporothrix mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol 45:3198–3206

Marimon R, Gene J, Cano J et al (2008) *Sporothrix luriei*: a rare fungus from clinical origin. Med Mycol 46:621–625

Mesa-Arango AC, Reyes-Montes MR, Perez-Mejia A et al (2002) Phenotyping and genotyping of *Sporothrix schenckii* isolates according to geographic origin and clinical form of sporotrichosis. J Clin Microbiol 40:3004–3011

Nascimento RC, Espíndola NM, Castro RA et al (2008) Passive immunization with monoclonal antibody against 70-kDa putative adhesin of *Sporothrix schenckii* induces protection in murine sporotrichosis. Eur J Immunol 38:3080–3089

Negrini TC, Ferreira LS, Alegranci P et al (2013) Role of TLR-2 and fungal surface antigens on innate immune response against *Sporothrix schenckii*. Immunol Invest 42:36–48

Negrini TC, Ferreira LS, Arthur RA et al (2014) Influence of TLR-2 in the immune response in the infection induced by fungus *Sporothrix schenckii*. Immunol Invest 43:370–390

Nemecek JC, Wüthrich M, Klein BS (2006) Global control of dimorphism and virulence in fungi. Science 312:583–588

Oda LM, Kubelka CF, Alviano CS et al (1983) Ingestion of yeast forms of *Sporothrix schenckii* by mouse peritoneal macrophages. Infect Immun 39:497–504

Polacheck I, Kwon-Chung KJ (1988) Melanogenesis in *Cryptococcus neoformans*. J Gen Microbiol 134:1037–1041

Previato JO, Gorin PAJ, Haskins RH et al (1979) Soluble and insoluble glucans from different types of the human pathogen *Sporothrix schenckii*. Exp Mycol 3:92–105

Rippon JW (1988) Sporotrichosis. In: Rippon JW (ed) Medical mycology. The W. B. Saunders Company, Philadelphia, PA, pp 325–352

Rivera-Rodríguez N, Rodríguez del Valle N (1992) Effects of calcium ions on the germination of *Sporothrix schenckii* conidia. J Med Vet Mycol 30:185–195

Rodrigues AM, Kubitschek-Barreira PH, Fernandes GF et al (2015) Immunoproteomic analysis reveals a convergent humoral response signature in the *Sporothrix schenckii* complex. J Proteomics 115:8–22

Rodríguez-Caban J, González-Velázquez W, Pérez-Sánchez L et al (2011) Calcium/calmodulin kinase 1 and its relation to thermotolerance and hsp90 in *Sporothrix schenckii*: an RNAi and yeast two-hybrid study. BMC Microbiol 11:162

Rodriguez-Del Valle N, Rosario M, Torres-Blasini G (1983) Effects of pH, temperature, aeration and carbon source on the development of the mycelial or yeast forms of *Sporothrix schenckii* from conidia. Mycopathologia 82:83–88

Romero-Martinez R, Wheeler M, Guerrero-Plata A et al (2000) Biosynthesis and functions of melanin in *Sporothrix schenckii*. Infect Immun 68:3696–3703

Ruiz-Baca E, Toriello C, Perez-Torres A et al (2009) Isolation and some properties of a glycoprotein of 70 kDa(gp70) from the cell wall *of Sporothrix schenckii* involved in fungal adherence to dermal extracellular matrix. Med Mycol 47:185–196

Ruiz-Baca E, Mora-Montes HM, López-Romero E et al (2011) 2D-immunoblotting analysis of *Sporothrix schenckii* cell wall. Mem Inst Oswaldo Cruz 106:248–250

Ruiz-Baca E, Hernández-Mendoza G, Cuéllar-Cruz M et al (2014) Detection of two immunoreactive antigens of the cell wall of *Sporothrix brasiliensis* and *S. globosa*. Diagn Microbiol Infect Dis 79:328–330

Sandoval-Bernal G, Barbosa-Sabanero G, Shibayama M et al (2010) Cell wall glycoproteins participate in the adhesion of *Sporothrix schenckii* to ephitelial cells. Mycopathologia 171: 251–259

Sassá MF, Saturi AET, Souza LF et al (2009) Response of macrophage toll-like receptor 4 to a *Sporothrix schenckii* lipid extract during experimental sporotrichosis. Immunology 128:301–309

Sassá MF, Souza LF, de Abreu-Ribeiro LC et al (2012) Immune response against *Sporothrix schenckii* in TLR-4-deficient mice. Mycopathologia 174:21–30

Serrano S, Rodríguez del Valle N (1990) Calcium uptake and eflux during the yeast to mycelium transition in *Sporothrix schenckii*. Mycopathologia 112:1–9

Sgarbi GDB, Ribeiro-Da Silva AJ, Carlos IZ et al (1997) Isolation of ergosterol peroxide and its reversion to ergosterol in the pathogenic fungus *Sporothrix schenckii*. Mycopathologia 139: 9–14

Szaniszlo P (1985) Fungal dimorphism with emphasis on fungi pathogenic for humans. Plenum Press, New York, pp 3–-67

Tachibana T, Matsuyama T, Mitsuyama M (1998) Characteristic infectivity of *Sporothrix schenckii* to mice depending on routes of infection and inherent fungal pathogenicity. Med Mycol 36: 21–27

Teixeira-Castelo PA, Alves de Castro R, Nascimento-Cícera R et al (2009) Cell Surface expression of adhesins for fibronectin correlates with virulence in *Sporothrix schenckii*. Microbiology 155:3730–3738

Travassos LR, de Souza W, Mendoza-Previato L et al (1977) Location and biochemical nature of surface components reacting with concanavalin A in different cell types of *Sporothrix schenckii*. Exp Mycol 1:293–305

Tsuboi R, Sanada T, Takamori K et al (1987) Isolation and properties of extracellular proteinases from *Sporothrix schenckii*. J Bacteriol 169:4104–4109

Valentin-Berrios S, González-Velázquez W, Pérez-Sánchez L et al (2009) Cytosolic phospholipase A2: a member of the signalling pathway of a new G protein α subunit in *Sporothrix schenckii*. BMC Microbiol 9:100

Valle-Avilés L, Valentin-Berrios S, González-Méndez RR et al (2007) Functional, genetic and bioinformatic characterization of a calcium/calmodulin kinase gene in *Sporothrix schenckii*. BMC Microbiol 7:107

Verdan FF, Faleiros JC, Ferreira LS (2012) Dendritic cells are able to differentially recognize *Sporothrix schenckii* antigens and promote Th1/Th17 response *in vitro*. Immunobiology 217: 788–794

Zhang Z, Hou B, Xin Y et al (2012) Protein profiling of the dimorphic pathogenic fungus, *Sporothrix schenckii*. Mycopathologia 173:1–11

Zhang Z, Hou B, Zheng F et al (2013) Molecular cloning, characterization and differential expression of a *Sporothrix schenckii* STE20-like protein kinase SsSte20. Int J Mol Med 31: 1343–1348

Chapter 4
Environmental Conditions and Fungal Pathogenicity

Alexander Batista-Duharte, Damiana Téllez Martínez, Diana Bridon da Graça Sgarbi, and Iracilda Zeppone Carlos

Abstract The different species of the so-called *Sporothrix* complex are environmental fungus found in soils, plants, water, decaying vegetables, and other outdoor environments. Although they have been isolated from diverse locations, including contaminated areas, few studies have addressed the influence of the environment on the virulence of these pathogens. However, some researches in *S. schenckii* and other similar pathogenic fungi, suggest that adverse conditions in the natural habitat can trigger the expression of different virulence factors that confer survival advantages both in the environment and in host tissues. In this chapter, we approach advances in understanding of the biology of *S. schenckii* and how environmental factors can modify its virulence.

Keywords *Sporothrix schenckii* • Environment • Fungal pathogenicity • Virulence factors

4.1 Introduction

The vast majority of pathogenic fungi for humans and animals are environmental fungi, which normally live saprophytically outside the human body and can cause infections in certain susceptible hosts. The host body resembles certain aspects of

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the outdoor spaces, and these fungi have most likely gained their pathogenic potential in environmental niches. In these diverse places, fungi acquire properties favoring the colonization of host tissue, such as the capacity to adhere to surfaces and form biofilms, to compete with other microorganisms, and to deal with changes in temperature, ultraviolet (UV) radiation, pH, osmolarity, and other physical, chemical, and biological stress factors. Thus, in nature or in animal hosts, fungal cells must respond efficiently to changing environmental conditions to survive (Pérez-Sánchez et al. 2010). The organisms belonging to the *Sporothrix schenckii* complex have extraordinarily complex and variable ecological niches. These fungi can use different signal transduction pathways to sense the environment and to adapt quickly to changing conditions (Rodriguez-Caban et al. 2011). Interestingly, evidence has suggested that *S. schenckii* can modify its virulence in response to certain environmental stressors (Téllez et al. 2014). Thus, environment–pathogen–host interplay has a structuring effect on the diversity, frequency, and distribution of *Sporothrix* species and sporotrichosis outbreaks (Rodrigues et al. 2014).

4.2 Reports of the Presence of *S. schenckii* in Different Environmental Conditions

The survival of fungi in their environmental niches depends on their ability to adapt to changing conditions. Agents of the fungal genus *Sporothrix* have been isolated in diverse environmental conditions, including indoor and outdoor surfaces (Cooke and Foter 1958; Dixon et al. 1991; Ulfig et al. 1996; Yazdanparast et al. 2013) and soils and other places contaminated with chemical pollutants (Ulfig 1994; Kacprzak and Malina 2005; Prenafeta-Boldú et al. 2006; Pečiulytė 2010; Chao et al. 2012), in pH levels that range widely from 2.2 to 12.5 (Tapia Noriega et al. 1993; Ferreira et al. 2009), as well as in floors of swimming pools (Staib 1983), urban water distribution systems (Doggett 2000; US EPA 2002), desiccated mushrooms (Kazanas 1987), fleas, ants, and horse hair (Carrada-Bravo and Olvera-Macías 2013). It has been proposed that extreme environments can select for certain stress-tolerant fungi and can drive their evolution toward acquiring pathogenic properties and virulence (Casadevall et al. 2003; Gostinčar et al. 2011).

4.3 Extreme Environmental Conditions Observed in the Ecological Niches of *S. schenckii* Complex

4.3.1 Physical Factors

Environmental microorganisms are habitually exposed to different physical factors, including extreme temperatures, salinity, sunlight, and drought. *S. schenckii* is able

to resist extreme conditions, such as very low temperatures (Pasarell and Mcginnis 1992; Mendoza et al. 2005) and extreme osmotic pressure (Castellani 1967; de Capriles et al. 1993; Mendoza et al. 2005; Ferreira et al. 2009) for several years. In the same way, there are evidences that *S. schenckii* is able to resist the influence of radiations. The exposition of *S. schenckii* to different levels of UV light resulted in a conserved viability. However, a high frequency of morphological changes was observed, such as smaller colonies or changes in shape, depending on the strain and UV dose (Torres-Guerrer and Arenas-López 1998). In addition, it was demonstrated that *S. schenckii* yeast cells exposed to gamma radiation remained viable up to 9.0 kGy; but the protein metabolism was strongly affected. In this report, 7.0 kGy of gamma radiation abolished the ability to produce infection but retained the viability, metabolic activity, and morphology (de Souza et al. 2011).

4.3.2 Chemical Contaminants

The chemical contamination of different environments is currently a worldwide problem. Many environmental bacteria and fungi have been shown to possess an ability to survive by adapting or mutating at high concentrations of toxic substances and can even use some of them as carbon source, participating in the biodegradation process (Cerniglia 1997; Prenafeta-Boldú et al. 2002). Several interesting reports have analyzed the capacity of *S. schenckii* to resist and even to metabolize different chemical compounds. Zeyer et al. observed that, of 160 microorganisms tested, only *S. schenckii* showed significant degradation of cyanuric acid under aerobic conditions. They found rapid degradation to carbon dioxide and ammonia (Zeyer et al. 1981a). This study generated a patent relating to a microbial process for the degradation of cyanuric acid in waste water containing cyanuric acid (Zeyer et al. 1981b). In addition, the effect of fungicides against several environmental pathogenic fungi was evaluated, and *S. schenckii* showed the greatest resistance to these products compared with other fungi (Morehart and Larsh 1967).

Other reports provide evidence for the ability of *S. schenckii* to resist and even degrade diverse hydrocarbons. The group of Prenafeta-Boldú isolated *S. schenckii*, together with other environmental microorganisms, from air biofilters exposed to hydrocarbon-polluted gas streams and even assimilated volatile aromatic hydrocarbons as the sole source of carbon and energy. The data in this report show that many volatile-hydrocarbon-degrading strains are closely related to the very restricted number of human–pathogenic fungal species causing severe mycoses, especially neurological infections, in immunocompetent individuals (Prenafeta-Boldú et al. 2006). Interestingly, other species belonging to the genus *Sporothrix* are intrinsically able to biodegrade different aromatic hydrocarbons. For example, *S. variecibatus* has been used for the biodegradation of gas-phase styrene (Rene et al. 2010), while *S. cyanescens* is able to transform high concentrations of pentachloronitrobenzene (Liévremont et al. 1996). Studies performed in our laboratory provide evidence that *S. schenckii* is able to grow in high concentrations of

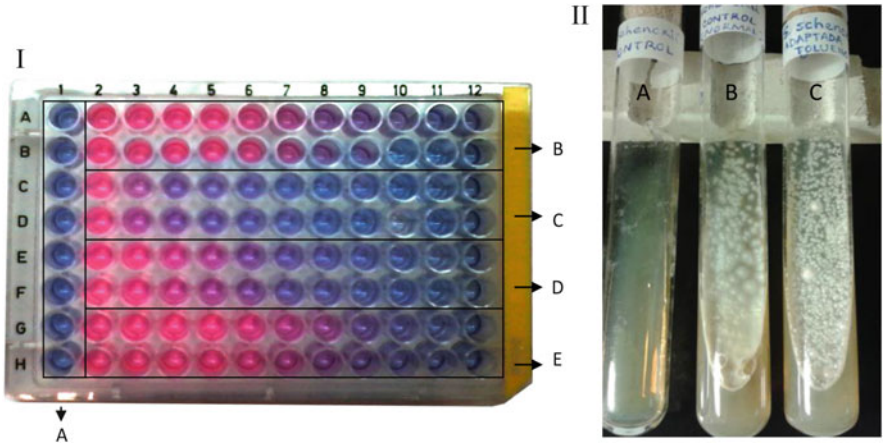

Fig. 4.1 (**I**) Viability test of *Sporothrix schenckii* ATCC 16345 pre-incubated for 24 h with three concentrations of toluene in brain-heart infusion (BHI) medium at 37 °C. The figure represents the fungal serial dilutions (duplicate) in RPMI 1640 medium post treatment with toluene. Viability was detected via the Alamar Blue method at 48 h of incubation at 37 °C. (A) Sterility control (negative); (B) Control without toluene. Inoculums of *S. schenckii* previously incubated with: (C) 100 g/L; (D) 50 g/L; (E) 10 g/L of toluene. (**II**) Growth of *S. schenckii* ATCC 16345 in different conditions: (A) Normal fungus incubated under closed saturated atmosphere of toluene; (B) Normal fungus growing in conventional conditions; (C) Fungus previously adapted to grow in high concentrations of toluene (100 g/L) (resistant strain) and cultured under closed saturated atmosphere of toluene. The solid culture media was Saboraud agar, and all were cultured at 35 °C

toluene, acquiring resistance mechanisms enabling it to copy with the saturated atmosphere of this contaminant (unpublished data) (Fig. 4.1).

Many fungi are able to grow in niches contaminated with toxic metals (Gray 1998; Zafar et al. 2007). *S. schenckii* has also been isolated in environments contaminated with heavy metals, suggesting they can be resistant to the toxic effects under field conditions (Ulfig et al. 1996; Kacprzak and Malina 2005). Some mechanisms have been proposed to explain the heavy metal tolerance in fungi, including extracellular sequestration with chelation and cell-wall binding, mainly employed in the avoidance of metal entry; the intracellular physical sequestration of metal by binding to metallothioneins (MTs), and the efflux from the cell via specific transporters (Fig. 4.2) (Valix and Loon 2003; Anahid et al. 2011; Samaranayake et al 2013; Jarosławiecka and Piotrowska-Seget 2014). Other mechanisms, specifically those associated with iron uptake in low-concentration environmental conditions and in the host, are discussed in Sect. 4.4.7.

4.3.3 Biological Factors

Diverse pathogenic fungi can interact with other microorganisms in their neighborhood. The in vitro interactions between colonies of *Blastomyces dermatitidis* and

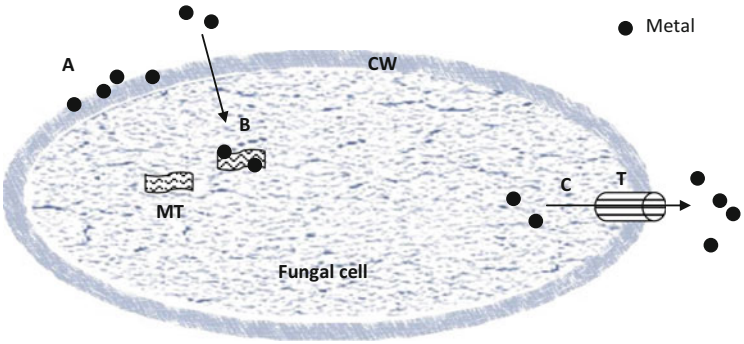

Fig. 4.2 Some mechanisms proposed to explain heavy metal tolerance in fungi (A) Adsorption on extracellular polysaccharides naturally occurring in the cell wall (CW) (extracellular sequestration); (B) Metals that gained access to the cytosol can be further inactivated by binding to the metallothioneins (MT); (C) Metals can be removed from de cytosol via transporters (T) (Efflux). (Based on Jarosławiecka and Piotrowska-Seget 2014; modified)

six other zoopathogenic fungi have been evaluated. The interactions were found to range from neutral with *Histoplasma capsulatum* and *Candida albicans* to strongly antagonistic with *Microsporum gypseum*, *Pseudallescheria boydii*, and *S. schenckii* and included lysis by *Cryptococcus neoformans* (Chaturvedi et al. 1988). In addition, Steenbergen et al. (2003) described how *C. neoformans* is able to interact with macrophages, slime molds, and amoebae, suggesting that fungal pathogenic strategies to avoid or survive to phagocytosis, may arise from environmental interactions with phagocytic microorganisms. Subsequently, these authors examined the interactions of three dimorphic fungi, *B. dermatitidis*, *H. capsulatum*, and *S. schenckii*, with the soil amoeba *Acanthameobae castellanii*. The ingestion of the yeast by this amoeba resulted in amoeba death and fungal growth, with an increase in hyphal cells (Steenbergen et al. 2004).

The biochemical events during phagocytosis by either *A. castellanii* or immune phagocytes are similar, suggesting that the "respiratory burst" enzyme(s) responsible for oxyradical generation in these two cell types is structurally related (Davis et al. 1991). In this way, soil amoebae may contribute to the selection and maintenance of pathogenic dimorphic fungi in the environment, conferring these microbes the capacity for virulence in mammals.

4.4 Fungal Virulence Factors Involved in Environmental Resistance and Pathogenicity

Virulence factors are molecules produced by pathogens and are involved in their pathogenicity by means of different mechanisms, including adhesion to cells, invasion and colonization of tissue in the host, obtaining nutrition from the host, evasion

of the host's immune response, and inhibition of the host's immune response, including microbicide mechanisms. According to Pirofski and Casadevall (2015), the "infectiveness," like virulence and the states in which microbes are found in hosts, is an outcome of host–microbe interaction that is a complex function of time and microbial, host, and environmental factors, independently and in combination.

The interaction of the dimorphic fungi with a mammalian host is not a requisite for fungal survival and virulence as occurs with other pathogenic microorganisms (Steenbergen et al. 2004). This phenomenon is called "ready-made" virulence (Casadevall et al. 2003), and it is possible that the environment contributes to the origin and maintenance of virulence in certain fungi (Wang and Casadevall 1994a, b; Rosas and Casadevall 1997, 2001; Taborda et al. 2008; Gessler et al. 2014; Téllez et al. 2014). Although many external influences are known to affect the pathogenicity of the *S. schenckii* complex, these influences and mechanisms have not been sufficiently studied in this complex. Nevertheless, the existence of common molecules interacting with environmental stressors described in diverse environmental fungus lead us to hypothesize that similar mechanisms may be acting in *S. schenckii*, for adaptation to these extreme conditions (Téllez et al. 2014). Several virulence factors in fungi can be produced for survival both in animal hosts and in the environment. This phenomenon is named "dual use" (Casadevall et al. 2003). Table 4.1 resumes several relevant mechanisms with "dual use" that can be used by *S. schenckii* to face extreme conditions in the environment and in the host (Téllez et al. 2014).

4.4.1 Genetic Polymorphism

Recent years have been important in the understanding of the molecular phylogeny of *S. schenckii* and have improved our knowledge of the taxonomy and epidemiology of this pathogenic fungus. Recent studies have shown differences in the virulence and drug resistance profiles among different species in the *S. schenckii* complex, concluding that *S. brasiliensis* is the most virulent species compared with *S. globosa* and *S. mexicana* (Fernández-Silva et al. 2012; Fernandes et al. 2013). In this way, suggestions have been made that emerging sporotrichosis is driven by clonal and recombinant *Sporothrix* species (Rodrigues et al. 2014).

Tests for susceptibility to antifungal drugs showed that *S. schenckii sensu stricto* and *S. brasiliensis* were highly susceptible to most of the antifungals tested in vitro compared with the more resistant *S. globosa* and *S. mexicana* species (Marimon et al. 2008; Rodrigues et al. 2014). Although the genomic organization and chromosome number of different species in the *S. schenckii* complex remain unknown, the karyotype profiles of *S. brasiliensis* isolates seems to be less variable than those observed in *S. schenckii* isolates (Sasaki et al. 2014). These results were consistent with phylogenetic data, where the variability among isolates within the species was less frequent in *S. brasiliensis* than in *S. schenckii s. str.* (Marimon et al. 2006; 2008; Rodrigues et al. 2013).

Table 4.1 Some virulence factors of *Sporothrix schenckii* complex with demonstrated or putative dual effects (protection against environmental and host stressors) (Updated from Téllez et al. 2014)

Attribute	Function		Selected references
	In the environment	In the host	
Genetic polymorphism	Generation of strain diversity to survive to environmental stress?	Antifungal drug resistance. Different virulence profile	Romeo and Criseo (2013)
Dimorphism	Mycelium morphology in its saprophytic phase	Yeast morphology in host tissues at 35–37 °C	Nemecek et al. (2006), Gauthier and Klein (2008)
Cell wall	Protects the cell from drastic changes in the external environment	Protects the cell from aggressive conditions in the host tissue	Oda et al. (1983), Carlos et al. (2003), Madrid et al. (2010), López-Esparza et al. (2013)
Melanin	Ultraviolet shielding, extreme temperature protection, reduced susceptibility to enzymatic degradation	Resistant to phagocytosis and oxidative killing by phagocytic cells. Antifungal drug resistance	Almeida-Paes et al. (2009), Romero-Martinez et al. (2000), Morris-Jones et al. (2003)
Ergosterol	Protection against oxidative killing by soil amoebas?	Protection against oxidative killing by phagocytic cells	Sgarbi et al. (1997)
Adhesins	In other fungi, adhesion genes are activated by diverse environmental triggers like carbon and/or nitrogen starvation or changes in pH or ethanol levels	Adhesion to the dermal and subendothelial matrix, transposition of the endothelial barrier, immunomodulators	Verstrepen et al. (2003), Sampermans et al. (2005), Figueiredo et al. (2007), Lima et al. (1999, 2001, 2004), Ruiz-Baca et al. (2008), Nascimento et al. (2008)
Proteinase	Nutritional function	Tissue damage; degradation of antibodies	Da Rosa et al. (2009), Monod et al. (2002)
Catalase	Protection against ROS of soil amoebas?	Protection against ROS of host phagocytes	Davis et al. (1991), Xiao-Hui et al. (2008)
Superoxide dismutase	Protection against oxygen-derived oxidants?	Intracellular growth	Pérez-Sánchez et al. (2010)
Nitroreductase	Tolerance to environmental contaminants?	Resistance to NO in phagocytes?	Stopiglia et al. (2013), Aviv et al. (2014)
Siderophores, glutathione-dependent ferric reductase (GSH-FeR)	Iron uptake in the environment	Iron uptake inside the host	Pérez-Sánchez et al. (2010), Zarnowski and Woods (2005))

(continued)

Table 4.1 (continued)

Attribute	Function		Selected references
	In the environment	In the host	
Metallothionein	Provide protection against metal toxicity. Are induced at high concentrations of metals	Protection against oxidative stress, antifungal resistance	Samaranayake et al. (2013), Maria et al. (2014), Schwartz et al. (2013)
SSG-1	Survival under conditions of stress and nutrient limitation inside the human host or the environment		Pérez-Sánchez et al. (2010)

Other studies have been performed to elucidate the relationships and differences among these species and their role in pathogenic manifestations via sequencing studies in the calmodulin-encoding gene in the different members of the *S. schenckii* complex, revealing wide diversity in the calmodulin gene (regulated by Ca^{+2}) of *S. schenckii s. str*. In contrast, *S. brasiliensis* and *S. globosa* appeared to be more homogenous, with low genetic diversity (Romeo et al. 2011). More studies are necessary to determine the role of different genes from the species of the *S. schenckii* complex in virulence, drug resistance, and environmental resistance.

4.4.2 Cell Wall

The cell wall of *S. schenckii* is composed principally of glucans, galactomannans, rhamnomannans, chitin, glycoproteins, glycolipids, and eventually different levels of melanin (Travassos et al. 1977; Travassos and Lloyd 1980; Lopes-Bezerra et al. 2006; López-Romero et al. 2011). This structure is the direct zone of contact with the environment and the host and, although more studies are necessary to investigate their detailed structure, it seems to be that a proper wall cell composition is required to support external stress and virulence (Carlos et al. 2003; Madrid et al. 2010).

4.4.3 Dimorphism

The dimorphic fungi are a group of important human pathogens that possess the ability to switch between mould and yeast in response to different external conditions. In the environment, they grow as mycelium that produces conidia or infectious spores, capable of converting to pathogenic yeasts when they are transmitted to humans or other susceptible mammalian hosts, producing an infectious process (Klein and Tebbets 2007). *S. schenckii* complex exhibits mycelium morphology in the environment, with different temperature grades and at 25 °C in laboratory

conditions, while in host tissues they have yeast morphology at 35–37 °C. Dimorphism in *S. schenckii* has been related to the ability to adapt to environmental changes and to yield an increased virulence (Nemecek et al. 2006; Gauthier and Klein 2008).

The heterotrimeric G proteins are receptors of environmental signals and they are involved in fungal dimorphism and pathogenicity. They interact with the cytosolic phospholipase A_2 ($cPLA_2$), participating in the control of dimorphism in this fungus (Valentín-Berríos et al. 2009). The role of the G proteins in the response to different external adverse conditions help to explain how this *S. schenckii* is able to survive under external stress (environmental and inside the host) (Valentín-Berríos et al. 2009; Pérez-Sánchez et al. 2010). Other mechanisms participating in the control of the dimorphism include calcium uptake and the Ca^{2+}/calmodulin-dependent protein kinase (CaMK) pathway (Serrano and Rodriguez-del Valle 1990; Rivera-Rodríguez and Rodríguez-del Valle 1992; Valle-Aviles et al. 2007; Rodriguez-Caban et al. 2011), the mitogen-activated protein kinase (MAPK) cascade and cyclic AMP (cAMP) signaling pathways ((Hou et al. 2013; Nemecek et al. 2006), the Ste20-related kinases through the MAPK pathways (Zhang et al. 2013) and the tumor-promoting agent and PKC activator, phorbol-12-myristate-13-acetate (PMA) (Colon-Colon and Rodriguez-del Valle 1993).

4.4.4 Melanin

Melanins are a ubiquitous class of high-molecular-weight negatively charged pigments that are present and play important roles throughout the plant and animal kingdoms (Morris-Jones et al. 2003). Several types of melanin are known to exist in the fungal kingdom, but the majority is derived from 1.8-dihydroxynaphthalene (DHN) and known as DHN-melanins. The biosynthetic pathway that catalyzes DHN has been termed the pentaketide pathway and resides primarily in various human pathogenic fungi, including *S. schenckii* (Romero-Martinez et al. 2000; Morris-Jones et al. 2003) (Fig. 4.3).

Melanin has the ability to protect microorganisms against a broad range of toxic insults, enabling the survival of fungi in the environment and during infection (Gómez and Nosanchuk 2003; Eisenman and Casadevall 2012). Melanization reduces susceptibility to enzymatic degradation, toxicity from heavy metals, UV and nuclear radiation, extremes of temperature, oxygen and nitrogen free radicals, which may afford the fungus protection against similar insults in the environment (Wang and Casadevall 1994a, b; Rosas and Casadewall 1997, 2001; Taborda et al. 2008; Gessler et al. 2014). Melanin also reduces the susceptibility of pathogenic fungi to different antifungal drugs (Nosanchuk and Casadevall 2006) and to different immune mechanisms in the host, especially phagocytosis (Steenbergen et al. 2004).

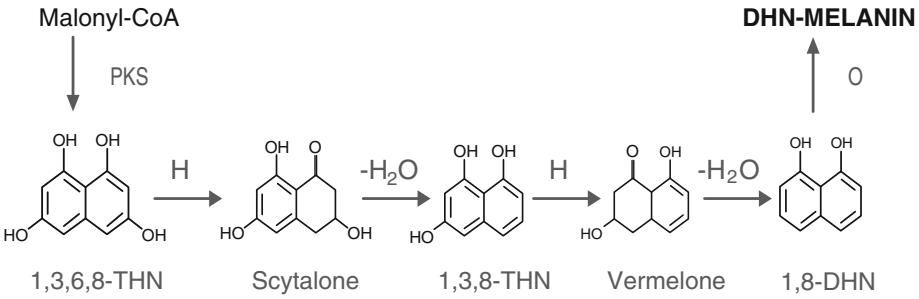

Fig. 4.3 Pentaketide pathway of melanin biosynthesis and some of the intermediate metabolites in *Sporothrix schenckii*. (O, oxidation; H, reduction; $-H_2O$, dehydration; PKS, polyketide synthase; 1,3,6,8-THN, 1,3,6,8-tetrahydroxynaphthalene; 1,3,6-THN, 1,3,6-trihydroxynaphthalene; 1,8-DHN, 1,8-dihydroxynaphthalene) (Adapted from Romero-Martinez et al. 2000 and Langfelder et al. 2003)

S. schenckii produces melanin or melanin-like compounds in vitro, and this pigment has a role in the pathogenesis of sporotrichosis (Morris-Jones et al. 2003). The melanin of *S. schenckii* has been found in the conidia, which are the infecting structures of this organism (Langfelder et al 2003). Romero-Martinez et al. (2000) studied the effects of two albino mutants of *S. schenckii*, mel10 and mel14, and found them to be more susceptible to UV irradiation, sodium nitrite, and hydrogen peroxide, with more sensitivity to phagocytosis than melanized cells. Interestingly, the addition of scytalone to the medium resulted in pigmented conidia and a complete restoration of resistance to phagocytosis and killing by human monocytes and murine macrophages, similar to melanized cells of the wild-type fungi, evidence that DHN-melanin is an important protective factor in the case of *S. schenckii* (Romero-Martinez et al. 2000).

The mechanism by which melanin interferes with phagocytosis is not clearly understood. It is possible that the surface charge plays a role, since melanins are charged polymers and phagocytosis is inversely correlated with cell charge (Wang et al. 1995; Walter et al. 1980). Moreover, melanin protects against the fungicide effect of reactive oxygen species (ROS) in the phagocytes (Wang and Casadevall 1994a,b). *S. schenckii* can also utilize phenolic compounds to augment melanin production, and this mechanism can participate in protection against external adverse conditions in both the environment and during infection (Almeida-Paes et al. 2009).

4.4.5 Ergosterol

Ergosterol (ergosta-5,7,22-trien-3β-ol) is a natural sterol found in the cell membranes of fungi, protozoa, lichens, and sponges and named for ergot, the common name of members of the fungal genus *Claviceps* from which ergosterol was first

isolated (Seitz and Pomeranz 1983; Baginski et al. 2002). Ergosterol is a useful target for antifungal drugs as it is present in the cell membranes of fungi, but not in those of animals, and many fungi and protozoa cannot survive without it. Amphotericin B, a classic antifungal drug, binds physically to ergosterol within the membrane, creating a polar pore in fungal membranes. This causes ions (predominantly potassium and protons) and other molecules to leak out, which kills the cell. However, because some of the functions and structures of ergosterol are similar to those of cholesterol in animal cells, adverse reactions during treatment with amphotericin B is a problem in clinical practice. Other antifungal drugs, such as itraconazole, miconazole, and clotrimazole, work in a different way, inhibiting the synthesis of ergosterol from lanosterol, and are less toxic (see Chap. 9).

In *S. schenckii*, ergosterol has an important role as part of the mechanism of the detoxification reaction of ROS. In the presence of reactive species of oxygen, ergosterol is transformed to its epidioxide ergosterol peroxide (5α,8-α-epidioxyergosta6,22- dien-3β-ol). Sgarbi et al. also showed that incubation of *S. schenckii* enzymatic extract with ergosterol peroxide resulted in its reversion to ergosterol. Such a reaction displayed by *S. schenckii* may enable it to elude the usual toxicity of ergosterol peroxide (a known reactive species) to fungal cells. On the other hand, the conversion and reversion properties shown by ergosterol could be correlated with cell mechanisms to maintain the lipid organization level of the membrane structures (Sgarbi et al. 1997).

Thus, it is conceivable that, in *S. schenckii*, ergosterol peroxide is formed as a protective mechanism to evade ROS during phagocytosis, and it may also represent a virulence factor in the host. Moreover, it is possible that this mechanism also has a protective role for the fungus in the external environment, facing the presence of amoebas living close to *S. schenckii* in soils. Interestingly, ergosterol is a pro-vitamin form of vitamin D_2, and exposure to UV light causes a chemical reaction that produces vitamin D_2 (Björn and Wang 2000). It is possible that this mechanism can contribute to the relative resistance of *S. schenckii* to UV radiation. Given this, more studies are necessary to determine the role of ergosterol as a protective mechanism from different environmental stresses such as radiation, chemical contamination, and other extreme conditions (Fig. 4.4).

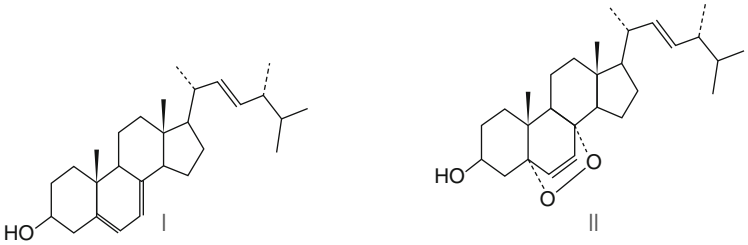

Fig. 4.4 Sterol structures isolated from *Sporothrix schenckii*. (**I**) Ergosterol; (**II**) Ergosterol peroxide

4.4.6 Adhesins

Adherence is an important capacity of a fungus for survival in different environments and for colonization and infection of the host. Some reports have indicated that *S. schenckii* is able to form biofilms in drinking water distribution systems (Doggett et al. 2000; EPA 2002). The development and maintenance of biofilms in such stressful conditions reflects a high capability for adaptation and resistance, as they had been (1) exposed to water flow and oligotrophic conditions for years, and (2) influenced by diverse abiotic factors, such as temperature, pH, and residual disinfectant (Siqueira et al. 2013).

Different adhesins mediate adherence to host tissues through interactions with some of the proteins that compose the extracellular matrix in the host. This is a crucial early process to establish an infection (Verstrepen and Klis 2006). The fungal adhesion is under tight transcriptional control by several interacting regulatory pathways. The adhesion genes are activated by diverse environmental triggers such as carbon and/or nitrogen starvation or changes in pH or ethanol levels (Verstrepen et al. 2003; Sampermans et al. 2005). The outer layer of the cell wall of *S. schenckii* contains several adhesins with a central role in host–pathogen interactions and is associated with the pathogenesis of sporotrichosis. It has been reported that *S. schenckii* adhered to fibronectin and laminin in soluble and immobilized forms (Lima et al. 1999, 2001, 2004) and that this is a key step for the transposition of the endothelial barrier (Figueiredo et al. 2007).

The 70 kDa glycoprotein (gp70) on the cell wall of *S. schenckii* mediates the adhesion of the fungus to the dermal and subendothelial matrix. This gp70, and another protein with a slightly lower molecular mass of 67 kDa, were detected from different isolates (Ruiz-Baca et al 2008; Nascimento et al. 2008; Teixeira et al. 2009). Sera obtained from patients with sporotrichosis reacted with 70 kDa antigens (Scott and Muchmore 1989). Moreover, hyperimmune sera of mice infected with *S. schenckii* also reacted with gp 70, and monoclonal antibodies specific for gp70 were protective against experimental infection (Nascimento et al. 2008). In addition, a reduced level of gp70 expression was found in virulent *S. brasiliensis* and *S. schenckii* strains, while a high expression of this molecule is associated with a lower virulence profile. All these reports provide evidence for the immunogenicity of gp 70 inducing a protective immune response in the host (Castro et al. 2013).

4.4.7 Enzyme Production

Diverse inducible enzymes are important virulence factors in pathogenic fungi. Nitroreductase is a member of a group of enzymes that reduces the wide range of nitroaromatic compounds and has potential industrial applications (Stopiglia

et al. 2013). Nitroreductase activity has been detected in a diverse range of bacteria and in yeast (Lee et al. 2008; Aviv et al. 2014), and it is widely distributed in the *S. schenckii* complex (Stopiglia et al. 2013). A recent study in an emergent *Salmonella enterica* serovar Infantis strain demonstrated that the fixation of adaptive mutations in the DNA gyrase (*gyrA*) and nitroreductase (*nfsA*) genes conferred resistance to quinolones and nitrofurans and contributes to the stress tolerance and pathogenicity of this bacteria (Aviv et al. 2014). The possible role of nitroreductase in the *S. schenckii* tolerance of adverse environmental conditions and in the host, the resistance to oxidative stress, antifungal drugs, and other aspects involved in pathogenicity, need to be studied.

Recent studies have investigated the activity of several enzymes related to fungal virulence of 151 Brazilian *S. schenckii* isolates from five different geographic regions of Brazil. All of the *S. schenckii* isolates presented urease and DNase activities. Only three (15.78 %) isolates (one from the north and two from the southeast regions) showed gelatinase activity, and five (26.31 %) isolates (one from the north, three from the northeast, and one from the southeast regions) showed proteinase activity of 0.68, 0.88, 0.85, 0.55, and 0.78, respectively. Additionally, only four (21.05 %) isolates (one from the north, one from the central west, and two from the southeast regions) showed caseinase activity of 0.75, 0.87, 0.89, and 0.87, respectively (Ferreira et al. 2009).

Another report of isolates from Venezuela confirmed the urease activity (Mendoza et al. 2005), while another study in India reported that all of the mycelial forms of *S. schenckii* could split urea (Ghosh et al. 2002). On the other hand, Fernandes et al. (2009) reported that, while the "highly virulent" *S. schenckii* isolates show a profile of secreted enzymes (proteinase, caseinase, gelatinase, DNase, and urease), most of these enzymes were not observed in the hypervirulent species *S. brasiliensis*. This observation indicates that the mechanisms promoting pathogenesis are much more complex, and can differ among closely related *Sporothrix* species (Romeo and Criseo 2013).

Iron is critical for fungal survival. Successful pathogens have developed mechanisms for iron acquisition and utilization in the face of environmental or host-mediated scarcity (Schaible and Kaufmann 2004). Almost all iron uptake by fungi involves reduction from the ferric to the ferrous form via two general mechanisms. The first is the synthesis of siderophores that chelate iron, which is ultimately taken up as a siderophore-iron complex (Kosman 2003). Unlike other fungi such as *S. cerevisiae* (Kaplan et al. 2006), *S. schenckii* is capable of producing its own siderophores in response to low iron availability (Holzberg and Artis 1983; Pérez-Sánchez et al. 2010). The second is that iron reduction can be catalyzed by specific membrane-associated or secreted enzymic proteins (iron reductases) or secreted external low-molecular-mass reductants. Zarnowski and Woods (2005) studied glutathione-dependent ferric reductase (GSH-FeR) activities in different dimorphic zoopathogenic fungal species, including *S. schenckii*, and provided evidence for a

role of this enzyme in extracellular reductive iron acquisition. These mechanisms can be involved in the pathogenicity and survival under conditions of environmental stress or inside the host.

4.5 Conclusion and Future Perspectives

Although more studies evaluating the influence of different environmental factors on the physiology and pathogenicity of the *S. schenckii* complex are necessary, all available data suggest the existence of strategies that pathogenic fungi acquire to survive the adverse environmental conditions. In turn, these mechanisms of acquired resistance provide the ability to infect animals and may further allow the emergence of opportunistic pathogens from these microenvironments (Baumgardner 2012).

Understanding of interactions between fungi and their potential hosts in the environment is in its infancy; however, initial observations suggest this will be an extremely rich area of investigation for exploring the fundamental questions of fungal pathogenesis (Casadevall et al. 2003; Prenafeta-Boldú et al. 2006). This knowledge will contribute to the design of new strategies for the control of sporotrichosis (Fig. 4.5).

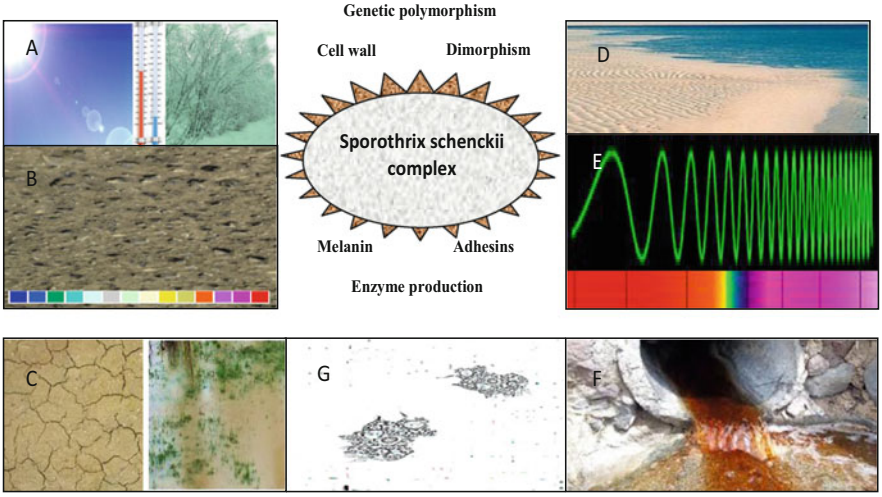

Fig. 4.5 Environmental stressor promotes *Sporothrix schenckii* virulence. The origin of virulence in *S. schenckii* should be related to interactions of the pathogen with different environmental challengers present in their natural habitat. (**a**) Extreme temperatures; (**b**) Extreme pH (basic/acid); (**c**) moisture/drought; (**d**) Salinity; (**e**) Radiation; (**f**) Chemical pollution; (**g**) Soil amoebas. These environmental stressors are resisted due to constitutive fungal structures and inducible molecules to acquire survival capacity and become virulence factors in the infected host

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References

Almeida-Paes R, Frases S, Fialho PCM, Gutierrez-Galhardo MC et al (2009) Growth conditions influence melanization of Brazilian clinical *Sporothrix schenckii* isolates. Microbes Infect 11(5):554–562

Anahid S, Yaghmaei S, Ghobadinejad Z (2011) Heavy metal tolerance of fungi. Scientia Iranica 18(3):502–508

Aviv G, Tsyba K, Steck N et al (2014) A unique megaplasmid contributes to stress tolerance and pathogenicity of an emergent Salmonella enterica serovar Infantis strain. Environ Microbiol 16(4):977–994

Baginski M, Resat H, Borowski E (2002) Comparative molecular dynamics simulations of amphotericin. B-cholesterol/ergosterol membrane channels. Biochim Biophys Acta 1567(1–2):63–78

Baumgardner DJ (2012) Soil-related bacterial and fungal infections. JABFM 25(5):734–744

Björn LO, Wang T (2000) Vitamin D in an ecological context. Int J Circumpolar Health 59(1): 26–32

Carlos IZ, Sgarbi DB, Santos GC et al (2003) *Sporothrix schenckii* lipid inhibits macrophage phagocytosis: involvement of nitric oxide and tumour necrosis factor-alpha. Scand J Immunol 57:214–220

Carrada-Bravo T, Olvera-Macías MI (2013) New observations on the ecology and epidemiology of *Sporothrix schenckii* and sporotrichosis. Rev Latinoamer Patol Clin 60(1):5–24

Casadevall A, Steenbergen JN, Nosanchuk JD (2003) 'Ready made' virulence and 'dual use' virulence factors in pathogenic environmental fungi — the *Cryptococcus neoformans* paradigm. Curr Opin Microbiol 6:332–337

Castellani A (1967) Maintenance and cultivation of the common pathogenic fungi of man in sterile distilled water. Further Researches. J Trop Med Hyg 70:181–184

Castro RA, Kubitschek-Barreira PH, Teixeira PA et al (2013) Differences in cell morphometry, cell wall topography and gp70 expression correlate with the virulence of *Sporothrix brasiliensis* clinical isolates. PLoS One 8(10), e75656

Cerniglia CE (1997) Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications inbioremediation. J Ind Microbiol Biotechnol 19:324–333

Chao HJ, Chan CC, Rao CY et al (2012) The effects of transported Asian dust on the composition and concentration of ambient fungi in Taiwan. Int J Biometeorol 56(2):211–219

Chaturvedi VP, Randhawa HS, Chaturvedi S et al (1988) *In vitro* interactions between *Blastomyces dermatitidis* and other zoopathogenic fungi. Can J Microbiol 34(7):897–900

Colon-Colon W, Rodriguez-del Valle N (1993) Studies on Phase Transitions in *Sporothrix schenckii:* Possible Involvement of Protein Kinase C. Dimorphic Fungi Biol Med 225–239

Cooke WB, Foter MJ (1958) Fungi in bedding materials. Appl Microbiol 6:169–173

Da Rosa D, Gezuele E, Calegari L et al (2009) Excretion-secretion products and proteases from live *Sporothrix schenckii* yeast phase. Immunological detection and cleavage of human IgG. Rev Inst Med Trop S Paulo 51(1):1–7

Davis B, Chattings LS, Edwards SW (1991) Superoxide generation during phagocytosis by *Acanthamoeba castellanii* similarities to the respiratory burst of immune phagocytes. J General Microbiol 137:705–710

de Capriles CC, Mata ES, Lander A et al (1993) Experimental pathogenicity of *Sporothrix schenckii* preserved in water (Castellani). Mycopathologia 122(3):129–133

de Souza CML, do Nascimento EM, Aparecida MR et al (2011) Gamma radiation effects on *Sporothrix schenckii* yeast cells. Mycopathologia 171:395–401

Dixon DM, Salkin IF, Duncan RA et al (1991) Isolation and characterization of *Sporothrix schenckii* from clinical and environmental sources associated with the largest U.S. Epidemic of Sporotrichosis. J Clin Microbiol 29(6):1106–1113

Doggett MS (2000) Characterization of fungal biofilms within a municipal water distribution system. Appl Environ Microbiol 66(3):1249–1251

Eisenman HC, Casadevall A (2012) Synthesis and assembly of fungal melanin. Appl Microbiol Biotechnol 93(3):931–940

U.S. Environmental Protection Agency (2002). Health risks from microbial growth and biofilms in drinking water distribution systems. Office of Ground Water and Drinking Water Standards and Risk Management Division 1200 Pennsylvania Ave., NW

Fernandes GF, Do Amaral CC, Sasaki A et al (2009) Heterogeneity of proteins expressed by Brazilian *Sporothrix schenckii* isolates. Med Mycol 47(8):855–861

Fernandes GF, Dos Santos PO, Rodrigues AM et al (2013) Characterization of virulence profile, protein secretion and immunogenicity of different *Sporothrix schenckii* sensu stricto isolates compared with *S. globosa* and *S. brasiliensis* species. Virulence 4(3):241–249

Fernández-Silva F, Capilla J, Mayayo E et al (2012) Virulence of *Sporothrix luriei* in a Murine Model of Disseminated Infection. Mycopathologia 173:245–249

Ferreira GF, Oliveira PS, Candida CA et al (2009) Characteristics of 151 Brazilian *Sporothrix schenckii* Isolates from 5 Different geographic regions of Brazil: A forgotten and re-emergent pathogen. Open Mycology J 3:48–58

Figueiredo CC, Deccache PM, Lopes-Bezerra LM et al (2007) TGF-b1 induces transendothelial migration of the pathogenic fungus *Sporothrix schenckii* by a paracellular route involving extracellular matrix proteins. Microbiology 153:2910–2921

Gauthier G, Klein BS (2008) Insights into Fungal Morphogenesis and Immune Evasion: Fungal conidia, when situated in mammalian lungs, may switch from mold to pathogenic yeasts or spore-forming spherules. Microbe Wash DC 3(9):416–423

Gessler NN, Egorova AS, Belozerskaya TA (2014) Melanin pigments of fungi under extreme environmental conditions (Review). Appl Biochem Microbiol 50(2):105–113

Ghosh A, Maity PK, Hemashettar BM et al (2002) Physiological characters of *Sporothrix schenckii* isolates. Mycoses 45:449–454

Gómez BL, Nosanchuk JD (2003) Melanin and fungi. Curr Opin Infect Dis 16(2):91–96

Gostinčar C, Grube M, Gunde-Cimerman N (2011) Evolution of fungal pathogens in domestic environments? Fungal Biol 115(10):1008–1018

Gray SN (1998) Fungi as potential bioremediation agents in soil contaminated with heavy or radioactive metals. Biochem Soc Trans 26(4):666–670

Holzberg M, Artis WM (1983) Hydroxamate siderophore production by opportunistic and systemic fungal pathogens. Infect Immun 40(3):1134–1139

Hou B, Zhang Z, Zheng F et al (2013) Molecular cloning, characterization and differential expression of DRK1 in Sporothrix schenckii. Int J Mol Med 31:99–104

Jarosławiecka A, Piotrowska-Seget Z (2014) Lead resistance in microorganisms. Microbiology 160:12–25

Kacprzak M, Malina G (2005) The tolerance and Zn 2+, Ba2+ and Fe 3+ accumulation by *Trichoderma atroviride* and *Mortierella exigua* isolated from contaminated soil. Canadian. J Soil Sci 85(2):283–290

Kaplan J, McVey Ward D et al (2006) Iron-dependent metabolic remodeling in *S. cerevisiae*. Biochim Biophys Acta 1763(7):646–651

Kazanas N (1987) Pathogenic fungi isolated from desiccated mushrooms, seaweed, anchovies and rice sticks imported from the Orient. J Food Protect 50:933–939

Klein BS, Tebbets B (2007) Dimorphism and virulence in fungi. Curr Opin Microbiol 10:314–319

Kosman DJ (2003) Molecular mechanisms of iron uptake in fungi. Mol Microbiol 47(5): 1185–1197

Langfelder K, Streibel M, Jahn B et al (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 38(2):143–158
Lee BU, Park SC, Cho YS et al (2008) Expression and characterization of the TNT nitroreductase of *Pseudomonas sp*. HK-6 in *Escherichia coli*. Curr Microbiol 56:386–390
Liévremont D, Seigle-Murandia DF, Benoit-Guyod JL (1996) Effects of culture parameters on pentachloronitrobenzene removal by *Sporothrix cyanescens*. Chemosphere 32:361–375
Lima OC, Figueiredo CC, Pereira BA et al (1999) Adhesion of the human pathogen *Sporothrix schenckii* to several extracellular matrix proteins. Braz J Med Biol Res 32:651–657
Lima OC, Figueiredo CC, Previato JO et al (2001) Involvement of fungal cell wall components in adhesion of Sporothrix schenckii to human fibronectin. Infect Immun 69:6874–6880
Lima OC, Bouchara JP, Renier G et al (2004) Immunofluorescence and flow cytometry analysis of fibronectin and laminin binding to *Sporothrix schenckii* yeast cells and conidia. Microb Pathog 37(3):131–140
Lopes-Bezerra LM, Schubach AO, Costa RO (2006) *Sporothrix schenckii* and sporotrichosis. An Acad Bras Cienc 78:293–308
López-Romero E, Reyes-Montes M, Pérez-Torres A et al (2011) *Sporothrix schenckii* complex and sporotrichosis, an emerging health problem. Future Microbiol 6(1):85–102
Madrid IM, Xavier MO, Mattei AS et al (2010) Role of melanin in the pathogenesis of cutaneous sporotrichosis. Microbes Infect 12:162–165
Maria VL, Ribeiro MJ, Amorim MJ (2014) Oxidative stress biomarkers and metallothionein in Folsomia candida–responses to Cu and Cd. Environ Res 133:164–169
Marimon R, Gené J, Cano J et al (2006) Molecular phylogeny of *Sporothrix schenckii*. J Clin Microbiol 44:3251–3256
Marimon R, Serena C, Gene J et al (2008) In vitro antifungal susceptibilities of five species of Sporothrix. Antimicrob Agents Chemother 52:732–734
Mendoza M, Alvarado P, Díaz de Torres E et al (2005) Physiological comportment and *in vitro* sensitivity of *Sporothrix schenckii* isolates maintained for 18 years by two preservation methods. Rev Iberoam Micol 22:151–156
Monod M, Capoccia S, Léchenne B et al (2002) Secreted proteases from pathogenic fungi. Int J Med Microbiol 292(5-6):405–419
Morehart AL, Larsh HW (1967) Laboratory examination of organic fungicides against zoopathogenic fungi in soil. Appl Microbiol 15(5):1248–1251
Morris-Jones R, Youngchim S, Gomez BL et al (2003) Synthesis of Melanin-Like Pigments by *Sporothrix schenckii* In vitro and during Mammalian Infection. Infect Immun 71(7):4026–4033
Nascimento RC, Espíndola NM, Castro RA et al (2008) Passive immunization with monoclonal antibody against 70-kDa putative adhesin of *Sporothrix schenckii* induces protection in murine sporotrichosis. Eur J Immunol 38:3080–3089
Nemecek JC, Wuthrich M, Klein BS (2006) Global control of dimorphism and virulence in fungi. Science 312:583–588
Nosanchuk JD, Casadevall A (2006) Impact of melanin on microbial virulence and clinical resistance to antimicrobial compounds. Antimicrob Agents Chemother 50:3519–3528
Oda LM, Kubelka CF, Alviano CS et al (1983) Ingestion of yeast forms of *Sporothrix schenckii* by mouse peritoneal macrophages. Infect Immun 39(2):497–504
Pasarell L, Mcginnis MR (1992) Viability of Fungal Cultures Maintained at -70°C. J Clin Microbiol 30(4):1000–1004
Pečiulytė D (2010) Effect of long-term industrial pollution on microorganisms in soil of deciduous forests situated along a pollution gradient next to a fertilizer factory. Species diversity and community structure of soil fungi. Ekologija 56(3-4):132–143
Pérez-Sánchez L, González E, Colón-Lorenzo EE et al (2010) Interaction of the heterotrimeric G protein alpha subunit SSG-1 of *Sporothrix schenckii* with proteins related to stress response and fungal pathogenicity using a yeast two-hybrid assay. BMC Microbiol 10:317
Pirofski LA, Casadevall A (2015) What is infectiveness and how is it involved in infection and immunity? BMC Immunol 16:13

Prenafeta-Boldú FX, Vervoort J, Grotenhuis JT et al (2002) Substrate interactions during the biodegradation of benzene, toluene, ethylbenzene, and xylene (BTEX)hydrocarbons by the fungus Cladophialophora sp. strain T1. Appl Environ Microbiol 68(6):2660–2665

Prenafeta-Boldú FX, Summerbell R, de Hoog GS (2006) Fungi growing onaromatic hydrocarbons: biotechnology's unexpected encounterwith biohazard? FEMS Microbiol Rev 30:109–130

Rene ER, Veiga MC, Kennes C (2010) Biodegradation of gas-phase styrene using the fungus *Sporothrix variecibatus:* impact of pollutant load andtransient operation. Chemosphere 79(2): 221–227

Rivera-Rodríguez N, Rodríguez-del Valle N (1992) Effects of calcium ions on the germination of *Sporothrix schenckii* conidia. J Med Vet Mycol 30(3):185–195

Rodrigues AM, de Melo Teixeira M et al (2013) Phylogenetic Analysis Reveals a High Prevalence of *Sporothrix brasiliensis* in Feline Sporotrichosis Outbreaks. PLoS Negl Trop Dis 7, e2281

Rodrigues AM, de Hoog GS, Zhang Y et al (2014) Emerging sporotrichosis is driven by clonal and recombinant Sporothrix species. Emerg Microbes Infect 3, e32

Rodriguez-Caban J, Gonzalez-Velazquez W, Perez-Sanchez L et al (2011) Calcium/calmodulin kinase1 and its relation to thermotolerance and HSP90 in *Sporothrix schenckii*: an RNAi and yeast two-hybrid study. BMC Microbiol 11:162

Romeo O, Criseo G (2013) What lies beyond genetic diversity in *Sporothrix schenckii* species complex? New insights into virulence profiles, immunogenicity and protein secretion in S. schenckii sensu stricto isolates. Virulence 4(3):1–4

Romeo O, Scordino F, Criseo G (2011) New Insight into Molecular Phylogeny and Epidemiology of *Sporothrix schenckii* Species Complex Based on Calmodulin-Encoding Gene Analysis of Italian Isolates. Mycopathologia 172:179–186

Romero-Martinez R, Wheeler M, Guerrero-Plata A et al (2000) Biosynthesis and functions of melanin in *Sporothrix schenckii*. Infect Immun 68(6):3696–3703

Rosas AL, Casadevall A (1997) Melanization affects susceptibility of *Cryptococcus neoformans* to heat and cold. FEMS Microbiol Lett 153:265–272

Rosas AL, Casadevall A (2001) Melanization decreases the susceptibility of *Cryptococcus neoformans* to enzymatic degradation. Mycopathologia 151:53–56

Ruiz-Baca E, Toriello C, Pérez-Torres A et al (2008) Isolation and some properties of a glycoprotein of 70 kDa (Gp70) from the cell wall of *Sporothrix schenckii* involved in fungal adherence to dermal extracellular matrix. Med Mycol 47:185–196

Samaranayake YH, Cheung BP, Wang Y et al (2013) Fluconazole resistance in *Candida glabrata* is associated with increased bud formation and metallothionein production. J Med Microbiol 62(Pt 2):303–318

Sampermans S, Mortier J, Soares EV (2005) Flocculation onset in *Saccharomyces cerevisiae*: the role of nutrients. J Appl Microbiol 98:525–531

Sasaki AA, Fernandes GF, Rodrigues AM et al (2014) Chromosomal Polymorphism in the *Sporothrix schenckii* complex. PLoS One 9(1):e86819

Schaible UE, Kaufmann SH (2004) Iron and microbial infection. Nat Rev Microbiol 2(12): 946–953

Schwartz JA, Olarte KT, Michalek JL et al (2013) Regulation of copper toxicity by *Candida albicans* GPA2. Eukaryot Cell 12(7):954–961

Scott EN, Muchmore HG (1989) Immunoblot analysis of antibody responses to *Sporothrix schenckii*. J Clin Microbiol 27(2):300–304

Seitz LM, Pomeranz Y (1983) Ergosterol, ergosta-4,6,8(14),22-tetraen-3-one, ergosterol peroxide and chitin in ergoty barley, rye and other grasses. J Agric Food Chem 31(5):1036–1103

Serrano S, Rodriguez-del Valle N (1990) Calcium uptake and efflux during the yeast to mycelium transition in *Sporothrix schenckii*. Mycopathologia 112(1):1–9

Sgarbi DB, da Silva AJ, Carlos IZ et al (1997) Isolation of ergosterol peroxide and its reversion to ergosterol in the pathogenic fungus *Sporothrix schenckii*. Mycopathologia 139(1):9–14

Siqueira VM, Oliveira HB, Santos C et al (2013) Biofilms from a Brazilian water distribution system include filamentous fungi. Can J Microbiol 59(3):183–188

Staib F (1983) Isolation of *Sporothrix schenckii* from the floor of an indoor swimming pool. Zentralbl Bakteriol Mikrobiol Hyg B 177(6):499–506

Steenbergen J, NosanchukN MSD, Casadevall A (2003) *Cryptococcus neoformans* virulence is enhanced after intracellular growth in the genetically malleable host *Dictyostelium discoideum*. Infect Immun 71:4862–4872

Steenbergen JN, Nosanchuk JD, Malliaris SD et al (2004) Interaction of *Blastomyces dermatitidis*, *Sporothrix schenckii*, and *Histoplasma capsulatum* with *Acanthamoeba castellanii*. Infect Immun 72(6):3478–3488

Stopiglia CD, Carissimi M, Daboit TC et al (2013) Application of 6-nitrocoumarin as a substrate for the fluorescent detection of nitroreductase activity in *Sporothrix schenckii*. Rev Inst Med Trop Sao Paulo 55(5):353–356

Taborda CP, da Silva MB, Nosanchuk JD et al (2008) Melanin as a virulence factor of *Paracoccidioides brasiliensis* and other dimorphic pathogenic fungi: a minireview. Mycopathologia 165(4-5):331–339

Tapia Noriega C, Rivera Garay R, Sabanero G et al (1993) Sporothrix schenckii: cultures in different soils. Rev Latinoam Microbiol 35(2):191–194

Teixeira PA, de Castro RA, Nascimento RC et al (2009) Cell surface expression of adhesins for fibronectin correlates with virulence in Sporothrix schenckii. Microbiology 155(Pt 11): 3730–3738

Téllez MD, Batista-Duharte A, Portuondo D et al (2014) *Sporothrix schenckii* complex biology: environment and fungal pathogenicity. Microbiology 160(Pt 11):2352–2365

Torres-Guerrer H, Arenas-López G (1998) UV irradiation induced high frequency of colonial variants with altered morphology in *Sporothrix schenckii*. Med Mycol 36(2):81–87

Travassos LR, Lloyd KO (1980) *Sporothrix schenckii* and related species of *Ceratocystis*. Microbiol Rev 44:683–721

Travassos LR, de Sousa W, Mendonça-Previato L et al (1977) Location and biochemical nature of surface components reacting with concanavalin A in different cell types of *Sporothrix schenckii*. Exp Mycol 1:293–305

Ulfig K (1994) The occurrence of keratinolytic fungi in the polluted environment of the Labedy District in Gliwice. Rocz Panstw Zakl Hig 45(4):337–346

Ulfig K, Terakowski M, Lukasik W (1996) A preliminary study on the occurrence of keratinolytic fungi in the street sweepings from Chorzów. Rocz Panstw Zakl Hig 47(2):143–149

Valentín-Berríos S, González-Velázquez W, Pérez-Sánchez L et al (2009) Cytosolic phospholipase A2: a member of the signalling pathway of a new G protein α subunit in *Sporothrix schenckii*. BMC Microbiol 9:100

Valix M, Loon LO (2003) Adaptive tolerance behavior of fungi in heavy metals. Miner Eng 16: 193–198

Valle-Aviles L, Valentin-Berrios S, Gonzalez-Mendez RR et al (2007) Functional, genetic and bioinformatic characterization of a calcium/calmodulin kinase gene in *Sporothrix schenckii*. BMC Microbiol 7:107

Verstrepen KJ, Klis FM (2006) Flocculation, adhesion and biofilm formation in yeasts. Mol Microbiol 60(1):5–15

Verstrepen KJ, Derdelinckx G, Verachtert H et al (2003) Yeast flocculation: what brewers should know. Appl Microbiol Biotechnol 61:197–205

Walter H, Graham LL, Krob EJ et al (1980) Correlation between phagocytic and membrane surface properties reflected by partitioning of human peripheral blood monocytes in two-polymer aqueous phases. Biochim Biophys Acta 602(2):309–322

Wang Y, Casadevall A (1994a) Decreased susceptibility of melanized *Cryptococcus neoformans* to UV light. Appl Environ Microbiol 60:3864–3866

Wang Y, Casadevall A (1994b) Susceptibility of melanized and *nonmelanized Cryptococcus neoformans* to nitrogen- and oxygen-derived oxidants. Infect Immun 64:3004–3007

Wang Y, Aisen P, Casadevall A (1995) Cryptococcus neoformans melanin and virulence: mechanism of action. Infect Immun 63(8):3131–3136

Xiao-Hui W, Ruo-Yu L, Cun-Wei C et al (2008) Differential mRNA expression of *Sporothrix schenckii* catalase gene in two growth phases and effect factors. Chin Med J (Engl) 121(20): 2100–2102

Yazdanparast SA, Dargahi H, Shahrokhi S et al (2013) Isolation and Investigation of Keratinophilic Fungi in the Parks of Municipality Districts of Tehran. Thrita J Med Sci 2(1):2–5

Zafar S, Aqil F, Ahmad I (2007) Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour Technol 98:2557–2561

Zarnowski R, Woods JP (2005) Glutathione-dependent extracellular ferric reductase activities in dimorphic zoopathogenic fungi. Microbiology 151(Pt 7):2233–2240

Zeyer J, Bodmer J, Hutter R (1981a) Rapid degradation of cyanuricacid by *Sporothrix schenckii*. Zentbl Bakteriol Hyg, Abt I Orig C 2:99–110

Zeyer J, Bodmer J, Hutter R (1981) Biodegradation with sporothrix fungi. PATENT US 4274955 A

Zhang Z, Hou B, Zheng F et al (2013) Molecular cloning, characterization and differential expression of a *Sporothrix schenckii* STE20-like protein kinase SsSte20. Int J Mol Med 31(6): 1343–1348

Chapter 5
Clinical Forms of Human Sporotrichosis and Host Immunocompetence

Maria Clara Gutierrez-Galhardo, Dayvison Francis Saraiva Freitas, and Antonio Carlos Francesconi do Valle

Abstract Sporotrichosis is a benign infection restricted to the skin and subcutaneous tissue. The disseminated cutaneous and extracutaneous forms are rare and associated with immunosuppression. In this chapter, we discuss the clinical presentation forms associated with sporotrichosis. Sporotrichosis in patients with human immunodeficiency virus/acquired immunodeficiency syndrome, pregnant women, and children are also described because of their clinical peculiarities.

Keywords Sporothrichosis • *Sporothrix schenckii* infection • Immunocompetence

5.1 Introduction

In most cases, sporotrichosis is a benign infection restricted to the skin, subcutaneous tissue, and the adjacent lymph vessels associated with the traumatic inoculation of soil, vegetables, or organic matter contaminated with *Sporothrix* spp. (Rippon 1988; Kwon-Chung and Bennett 1992; Lacaz 2002; Zancopé-Oliveira et al. 2011). The disseminated cutaneous and extracutaneous forms appear in less than 5 % of cases and arise after the hematogenous spread of the fungus and/or contiguity with a skin lesion or direct inoculation of the skin. On rare occasions, conidial inhalation produces granulomatous pneumonitis. Immunosuppression caused by conditions such as diabetes, alcoholism, malignancy, steroid therapy, chronic obstructive pulmonary disease, and acquired immunodeficiency syndrome (AIDS) creates a predisposition to these unusual forms of the mycosis (Rippon 1988; Kwon-Chung and Bennett 1992; Lacaz 2002).

Sporotrichosis affects both sexes and can occur at any age. Some animals have also been implicated in the transmission of sporotrichosis, including domestic cats, which are considered to be the most epidemiologically important in some regions

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(Barros et al. 2004). The risk for inter-human transmission is considered very rare, and only one case has been reported to date (Jin et al. 1990).

The disease can be classified into four clinical forms (Sampaio et al. 1954, cited in Ramos-e-Silva et al. 2007). The lymphocutaneous form is the most common presentation, accounts for up to 75 % of cases, and is the easiest form of sporotrichosis to diagnose. The lesions are usually located on the upper extremities and are characterized by the appearance of a primary lesion at the site of inoculation after 2 or 3 weeks. According to the time of evolution, this lesion can be ulcerated with an infiltrated base or a papular, nodular, nodular-ulcerative, ulcerative-gummy, or vegetative plaque. From the initial injury, it forms a chain of painless nodules along the path of the lymph vessels, known as sporotrichoid spread (Fig. 5.1). These nodules may soften and ulcerate with some exudate. Usually, the regional lymph nodes are not involved and no skin changes are observed between the nodes. Erythema can be present, but the pain is usually mild. A secondary infection may occur and is associated with increased pain, erythema, and suppuration.

The fixed cutaneous form is the second most common and accounts for approximately 20 % of cases. The lesion remains confined to the inoculation site, and the lymph vessels are not involved. The lesions are ulcers or verrucous, infiltrative, or ulcero-infiltrative plaques (Fig. 5.2). Small satellite lesions are common and are frequently observed in children. In addition to these lesions, a variety of presentations lead to a large number of differential diagnoses, such as nodules, tubercles, papules, pustules, cysts, and gummas. This clinical form is believed to occur as a result of prior sensitization of the individual to the fungus, especially in endemic areas, which results in better host immune control, thereby limiting the lesion. The most common sites for lesions are the upper and lower extremities, but the face, neck, and trunk can also be affected. Children present with a high index of lesions on the face. As in the lymphocutaneous form, patients with the fixed cutaneous form do not present with systemic symptoms. If left untreated, however, the lesions may evolve into a chronic course, or a spontaneous involution of the lesion can occur.

The disseminated cutaneous form presents with papular, nodular, infiltrative plaque, verrucous, gummy, ulcerated or ulcerous crusty disseminated lesions,

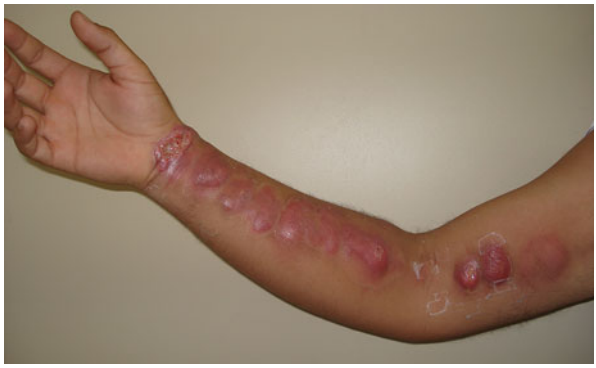

Fig. 5.1 The lymphocutaneous form of sporotrichosis. From the initial injury, it forms a chain of painless nodules along the path of the lymph vessels, known as sporotrichoid spread

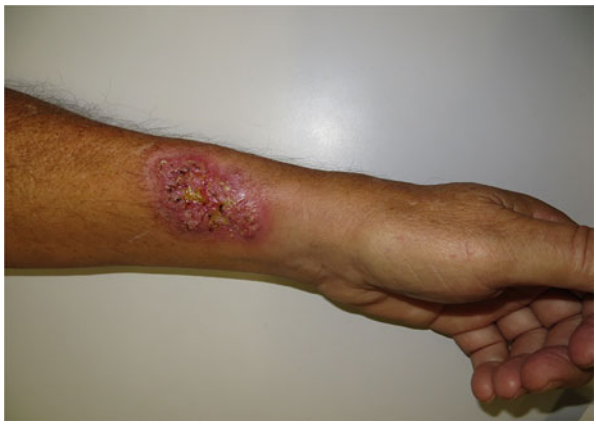

Fig. 5.2 The fixed cutaneous form of sporotrichosis manifested as an ulcero-infiltrative plaque on the forearm

sometimes coexisting on a single patient. Following inoculation of the skin, there is hematogenous dissemination, which initially presents as softened subcutaneous lesions that can ulcerate after weeks or months. Although quite rare, it has been reported in patients with AIDS, even as a first manifestation of this syndrome, as well as in patients receiving long courses of steroid therapy. Disseminated cutaneous sporotrichosis has been more frequently observed (up to 16 % of cases) in endemic zoonotic sporotrichosis. In these cases, the involvement of non-immunosuppressed patients has been attributed to multiple inoculations by domestic cats (Barros et al. 2004; Freitas et al. 2010).

The extracutaneous sporotrichosis form may present as unifocal or multifocal disease. Any organ or tissue can be affected. The symptoms are specific to the organ involved and are followed by fever and general commitment in some cases. Unifocal extracutaneous sporotrichosis usually involves cavitary disease of the lungs or arthritis; multifocal disease often involves joints, bones, and skin.

Osteoarticular sites are the most commonly affected sites after the skin. The infection is usually secondary to hematogenous spread or through local inoculation. Osteomyelitis, isolated or with arthritis, may be present with or without skin lesions. The most commonly affected bones are the tibia, the small bones of the feet and hands, the radius, the ulna, the skull, and the face. Patients may present with localized swelling and local sinus tract formation. Radiographic findings in osseous sporotrichosis may reveal small granulomas to large lytic lesions, similar to bacterial osteomyelitis, with destruction of the bone, periosteal reaction, and periarticular osteopenia, the loss of articular cartilage, and cystic changes. Bone destruction may extend into adjacent joints. These radiographic changes are not specific to sporotrichosis and may be seen in other fungal infections, tuberculosis, and acute bacterial osteomyelitis. The diagnosis of monoarthritis caused by *Sporothrix* spp. is often delayed because of its rarity and the lack of clinically typical cutaneous lesions. In general, the outcome is unfavorable with regard to joint function and is partly because of poor host response. Patients often present

with a hot joint that is swollen and tender and may have an effusion. The knee is the most commonly affected joint, but hand, elbow, and ankle joints may also be involved. Destructive arthritis or tenosynovitis may occur. These patients generally have limited systemic symptoms. Radiographic features are not diagnostic and are not pathognomonic. Osteoporosis, soft tissue swelling, articular cartilage erosion, and effusions are the most common findings. Similarly, tenosynovitis isolated or associated with osteoarticular sporotrichosis may appear.

Pulmonary infection is mainly caused by the inhalation of conidia from the environment and is classified as primary. However, when dissemination of the fungus to the lungs is hematogenous or lymphatic, the infection is called secondary or multifocal. Primary pulmonary sporotrichosis can be asymptomatic, may manifest as a chronic pulmonary disease with cavitation or with consolidation and reticulonodular infiltrates. Patients with multifocal sporotrichosis and pulmonary involvement present with multilobar reticulonodular infiltrates and rarely with cavitation. Symptoms in both forms of presentation are similar to those seen in other pulmonary mycoses, tuberculosis, and sarcoidosis, with fever, cough, anorexia, dyspnea, and malaise. A review of 86 cases reported between 1960 and 2010 confirmed these findings. The authors recorded 64 primary pulmonary and 22 multifocal cases and demonstrated an association between hemoptysis and primary cavitary presentation (Aung et al. 2013).

Ocular infection can result from an exogenous infection or hematogenous dissemination. It may manifest as conjunctivitis with characteristic visible granulomas, episcleritis, dacryocystitis (Fig. 5.3) (Freitas et al. 2014b), corneal ulceration, uveitis, nodular iritis, retrobulbar lesion, panophthalmitis, ulceration, or ectropia and can lead to total blindness in rare cases. Cases of isolated granulomatous conjunctivitis have been reported in Brazil without cutaneous disease and are related to cat zoonotic transmission (Barros et al. 2004).

Silva-Vergara et al. (2012) reported the occurrence of endocarditis, bilateral endophthalmitis, and lymphatic involvement due to sporotrichosis from *S. brasiliensis* in a patient infected with HIV. This patient underwent cardiac surgery to replace the mitral valve, with a favorable outcome, but developed bilateral blindness.

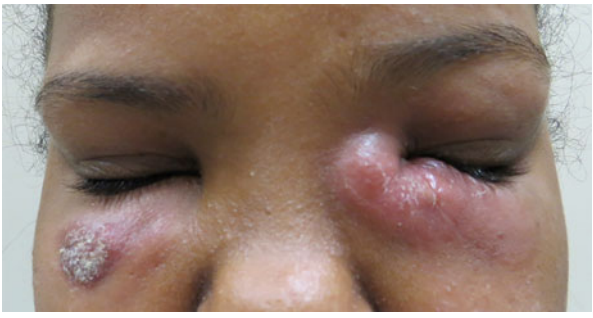

Fig. 5.3 Sporotrichosis presenting as acute dacryocystitis in a girl with associated cutaneous lesions

The mucosa of the mouth, pharynx, larynx, and nose can be infected by both direct and hematogenous routes. Enanthema, ulceration, suppuration, and vegetation are clinical manifestations. Signs and symptoms may include rhinorrhea, nasal obstruction, odynophagia, and dysphagia.

Infection of the nervous system presents as subacute meningoencephalitis in patients with disseminated forms of the disease; it is associated with a worse prognosis because of the difficulty of sterilizing the cerebrospinal fluid (CSF) with antifungal treatment. Brain abscesses may be present. Hydrocephalus is a potentially serious complication involving neurosporotrichosis and is often the cause of death. Clinical presentations are fever, headache, vomiting, lethargy, and seizures. CSF abnormalities are consistent with aseptic/clear fluid meningitis with hypoglycorrhachia, hyperproteinorrhachia, and low mononuclear cell counts. Brain computed tomography scans and magnetic resonance are helpful in diagnosing these cases.

5.2 Differential Diagnosis

Lymphocutaneous sporotrichosis is fairly common and can be confidently diagnosed. However, pyoderma, atypical *Mycobacterium* and *Nocardia* infection as well as leishmaniasis must also be considered (Coura 2005).

The clinical symptoms of the fixed form can be similar to those of pyoderma, paracoccidioidomycosis, chromoblastomycosis, cutaneous tuberculosis, atypical mycobacteria, tertiary syphilis, leishmaniasis, and even skin cancer.

Lesions of the disseminated cutaneous form can be confused with other deep mycosis, such as paracoccidioidomycosis, cryptococcosis or histoplasmosis, or with atypical mycobacteria and noninfectious granulomatous diseases.

The disseminated extracutaneous forms are diagnosed differently according to the affected organ. Other fungal conjunctivitis, bacterial, viral, or fungal meningitis, bacterial osteomyelitis, pulmonary fungal infections, tuberculosis, and sarcoidosis should also be considered.

5.3 Associated Clinical Conditions

Recently, associated clinical conditions throughout the course of sporotrichosis have been reported (Gutierrez-Galhardo et al. 2002, 2005; Barros et al. 2004; Orofino-Costa et al. 2010; Freitas et al. 2012b). In addition to general symptoms such as fever, malaise, headache, and asthenia, some patients present with signs and symptoms of hypersensitivity to the fungus (Barros et al. 2004; Freitas et al. 2010). Hypersensitivity reactions described to date that are attributed to sporotrichosis include erythema nodosum (Gutierrez-Galhardo et al. 2002), erythema multiforme (Fig. 5.4) (Gutierrez-Galhardo et al. 2005), reactive arthritis (Barros et al. 2004;

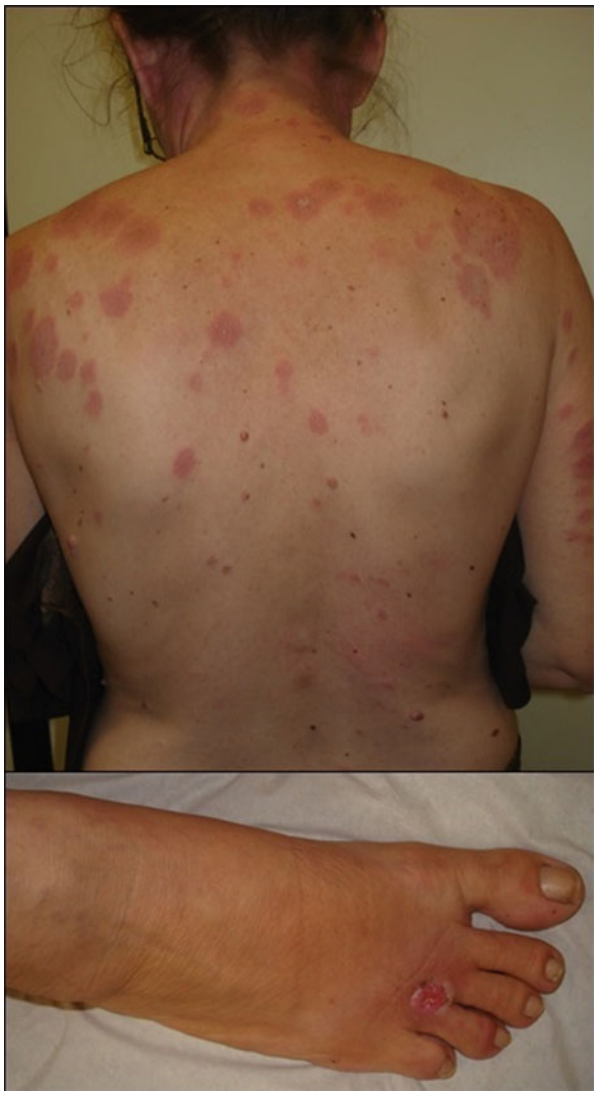

Fig. 5.4 Erythema multiforme on the dorsum of a patient with fixed cutaneous sporotrichosis on the foot

Orofino-Costa et al. 2010), and Sweet syndrome (Freitas et al. 2012b). The histopathology of the lesions of hypersensitivity exhibits a reactive infiltrate, and the search for the causative agent is always negative (Gutierrez-Galhardo et al. 2002, 2005). In cases of reactive arthritis, imaging tests are not compatible with infectious joint damage, and the symptoms resolve with the treatment of the mycosis (Orofino-Costa et al. 2010).

5.3.1 *Sporotrichosis in HIV/AIDS Patients*

Lipstein-Kresch et al. (1985) reported the first case of sporotrichosis and HIV co-infection, and drew attention to the opportunistic behavior of *Sporothrix*. Since then, some other reports have been published from different countries, mainly from the USA and Brazil.

Freitas et al. (2012a) described the largest case series of sporotrichosis in HIV-infected patients of 21 individuals. This study revealed a broad clinical spectrum of sporotrichosis, ranging from localized forms in patients without clinically defined AIDS (no clinical signs of immunodeficiency) to disseminated and severe disease leading to death. Large and numerous ulcerated (Fig. 5.5) nodular and cystic lesions as well as septum perforation have been observed in AIDS patients. The number of cases with CNS involvement was the same as for osteoarticular sporotrichosis, and the authors called attention to the neurotropism of *Sporothrix* spp. and the need for the systematic investigation of possible CNS involvement in patients with AIDS. Osteoarticular involvement was not associated with the inoculation site and was characterized by phalanx and knee osteomyelitis and tenosynovitis. In another study, AIDS patients presented with a higher index of disseminated disease associated with a low $CD4^+$ count and evolved with more severe clinical pictures, hence an increased need for hospitalization and risk of death (Freitas et al. 2014a). The relevance of this finding is that sporotrichosis

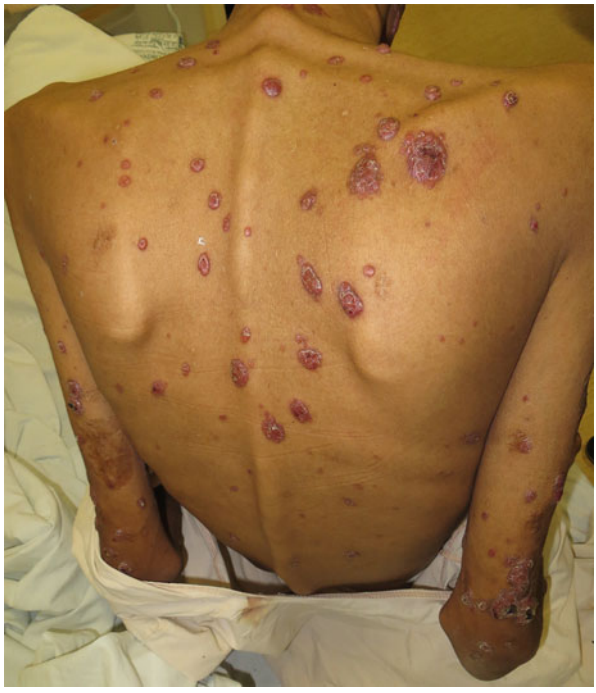

Fig. 5.5 The disseminated cutaneous form of sporotrichosis. Papular, infiltrative plaque lesions can be observed on the back of a patient with AIDS

should be considered as an opportunistic infection associated with AIDS in countries where this mycosis occurs.

5.3.2 Sporotrichosis in Pregnancy

Pregnant women are prone to the same manifestations and presentations of sporotrichosis as the general population, and no immune conditions specific to this group have been associated with different outcomes or prognoses. Two different case reports from the hyperendemic region of Rio de Janeiro, Brazil, demonstrated favorable outcomes for women with sporotrichosis during pregnancy (Orofino-Costa et al. 2011; Ferreira et al. 2012). A total of 17 women were diagnosed with localized cutaneous forms of sporotrichosis and all the 15 treated patients were cured. At first, there is no risk of the infection disseminating to the fetus in the localized forms of the disease. To date, whether a risk of vertical transmission in pregnant women with disseminated forms exists is unknown.

5.3.3 Sporotrichosis in Children

Children are affected differently according to their exposure level. In Peru, Brazil, and China large studies of children with sporotrichosis have been conducted. In Peru, no mechanisms of transmission were identified, and 60 % of the 238 patients reported were children aged <15 years (Pappas et al. 2000). In Brazil, the case studies mainly came from the south and southeast regions and affected children. Particularly in the south-east of Rio de Janeiro, cases are involved in the context of the zoonotic transmission by domestic cats (Barros et al. 2008). In China, the infection occurs mainly in the northeast rural area and involves the handling of contaminated plants (Song et al. 2013). Something very particular to clinical presentation in children is that the face represents the first or the second most frequent site of infection (after the upper limbs), an occurrence higher than that observed for adults, in whom the face comes after the lower limbs and trunk in almost all of the case series. This switch in the anatomical site of preference is most likely due to the thinner, more delicate skin in this area of the body of children (Rosa et al. 2005). Other differences are the high numbers of dacryocystitis (Freitas et al. 2014b) and the fixed cutaneous form in children, which is higher than in adults in some case series (Song et al. 2013). Some authors also experienced a faster rate for cure in younger populations (Song et al. 2013).

5.3.4 *Zoonotic Sporotrichosis*

The recognition of the new *Sporothrix* species of clinical interest (Marimon et al. 2007) has caused an important issue, among others, concerning the clinical and therapeutic relevance for this new classification of the causative agent of sporotrichosis. Not much is known at present, but the first studies dedicated to discussing the diversity of clinical presentations related to the different species conclude that *S. globosa* is associated with localized forms of the disease, a fact partially attributed to its inability to grow under high temperatures, notably above 35 °C (Marimon et al. 2007). Meanwhile, *S. brasiliensis* has been associated with hypersensitivity reactions, specifically in the region of Rio de Janeiro, Brazil, where this species has been implicated in the maintenance of the hyperendemic levels of zoonotic sporotrichosis. A trend in the association of this species with disseminated disease in patients without an immunosuppressive underlying cause has also been observed, as well as a good clinical response to oral itraconazole (Almeida-Paes et al. 2014).

5.4 Conclusions and Future Perspectives

Although sporotrichosis now causes morbidity worldwide, it is only rarely associated with mortality. However, the chronicity, the long-lasting and adverse effects associated with treatment, the increase in cases in certain risk groups, and the tendency to rapidly disseminate to different geographical areas, with reports of frequent outbreaks, is concerning health authorities. More collaborative research is necessary to clarify factors determining the current situation of the disease, looking for new diagnostic and therapeutic tools.

References

Almeida-Paes R, Oliveira MME, Freitas DFS et al (2014) Sporotrichosis in Rio de Janeiro, Brazil: *Sporothrix brasiliensis* is associated with atypical clinical presentations. PLoS Negl Trop Dis 8(9):e3094

Aung AK, Teh BM, McGrath C et al (2013) Pulmonary sporotrichosis: case series and systematic analysis of literature on clinico-radiological patterns and management outcomes. Med Mycol 51:534–544

Barros MBL, Schubach AO, Valle ACF et al (2004) Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: description of a series of cases. Clin Infect Dis 38:529–535

Barros MBL, Costa DLMA, Schubach TMP et al (2008) Endemic of zoonotic sporotrichosis: profile of cases in children. Pediatr Infect Dis J 27(3):246–250

Coura JR (2005) Dinâmica das Doenças Infecciosas e Parasitárias. Guanabara Koogan, Rio de Janeiro, pp 1161–1171

Ferreira CP, do Valle AC, Freitas DF et al (2012) Pregnancy during a sporotrichosis epidemic in Rio de Janeiro, Brazil. Int J Gynaecol Obstet 117:294–295

Freitas DFS, Valle ACF, Almeida-Paes R et al (2010) Zoonotic sporotrichosis in Rio de Janeiro, Brazil: a protracted epidemic yet to be curbed. Clin Infect Dis 50:453

Freitas DF, de Siqueira Hoagland B, do Valle AC et al (2012a) Sporotrichosis in HIV-infected patients: report of 21 cases of endemic sporotrichosis in Rio de Janeiro, Brazil. Med Mycol 50:170–178

Freitas DF, Valle AC, Cuzzi T et al (2012b) Sweet syndrome associated with sporotrichosis. Br J Dermatol 166:212–213. doi:10.1111/j.1365-2133.2011.10496.x

Freitas DFS, do Valle ACF, da Silva MBT et al (2014a) Sporotrichosis: an emerging neglected opportunistic infection in hiv-infected patients in Rio de Janeiro, Brazil. PLoS Negl Trop Dis 8 (8):e3110

Freitas DFS, Lima IAR, Curi CL et al (2014b) Acute dacryocystitis: another clinical manifestation of sporotrichosis. Mem Inst Oswaldo Cruz 109:262–264

Gutierrez-Galhardo MC, Schubach AO, Barros MBL et al (2002) Erythema nodosum associated with sporotrichosis. Int J Dermatol 41:114–116

Gutierrez-Galhardo MC, Barros MBL, Schubach AO et al (2005) Erythema multiforme associated with sporotrichosis. J Eur Acad Dermatol Venereol 19:507–509

Jin XZ, Zhang HD, Hiruma M et al (1990) Mother-and-child cases of sporotrichosis infection. Mycoses 33:33–36

Kwon-Chung KJ, Bennett JE (1992) Medical mycology, 2nd edn. Lea & Febiger, Philadelphia, pp 707–729

Lacaz CS (2002) Esporotricose e outras micoses gomosas. In: Lacaz CS, Porto E, Martins JEC, Heins-vaccari EM, Melo NT (eds) Tratado de Micologia Médica Lacaz, 9th edn. Sarvier, São Paulo, pp 479–497

Lipstein-Kresch E, Isenberg HD, Singer C et al (1985) Disseminated *Sporothrix schenckii* infection with arthritis in a patient with acquired immunodeficiency syndrome. Rheumatol 12:805–808

Marimon R, Cano J, Gene J et al (2007) *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol 45:3198–3206

Orofino-Costa R, Boia MN, Magalhães GA et al (2010) Arthritis as a hypersensitivity reaction in a case of sporotrichosis transmitted by a sick cat: clinical and serological follow up of 13 months. Mycoses 53:81–83

Orofino-Costa R, Bernardes-Engemann AR, Azulay-Abulafia L et al (2011) Sporotrichosis in pregnancy: case reports of 5 patients in a zoonotic epidemic in Rio de Janeiro, Brazil. An Bras Dermatol 86(5):995–998

Pappas PG, Tellez I, Deep AE et al (2000) Sporotrichosis in Peru: description of an area of hyperendemicity. Clin Infect Dis 30:65–70

Ramos-e-Silva M, Vasconcelos C, Carneiro S et al (2007) Sporotrichosis. Clin Dermatol 25:181–187

Rippon JW (1988) Medical mycology: the pathogenic fungi and the pathogenic actinomycetes, 3rd edn. WB Saunders, Philadelphia, pp 325–352

Rosa AC, Scroferneker ML, Vettorato R et al (2005) Epidemiology of sporotrichosis: a study of 304 cases in Brazil. J Am Acad Dermatol 52:451–459

Sampaio SAP, Lacaz CS, Almeida F (1954) Aspectos clínicos da esporotricose em São Paulo. Rev Hosp Clin Fac Med Sao Paulo 9(6):391–402

Silva-Vergara ML, de Camargo ZP, Silva PF et al (2012) Disseminated *Sporothrix brasiliensis* infection with endocardial and ocular involvement in an HIV-infected patient. Am J Trop Med Hyg 86(3):477–480

Song Y, Li SS, Zhong SX et al (2013) Report of 457 sporotrichosis cases from Jilin province, northeast China, a serious endemic region. J Eur Acad Dermatol Venereol 27(3):313–318

Zancopé-Oliveira RM, Almeida-Paes R, Oliveira MME et al (2011) New diagnostic applications in sporotrichosis. In: Khopkar U (ed) Skin biopsy perspectives. In Tech, Rijeka, pp 53–72

Chapter 6
Sporotrichosis in Animals: Zoonotic Transmission

Sandro Antonio Pereira, Isabella Dib Ferreira Gremião, and Rodrigo Caldas Menezes

Abstract Sporotrichosis is regarded as an emergent zoonosis, particularly in Brazil, where cats are important reservoirs. The disease is reported in different animal species, including cats, dogs, armadillos, horses, mules, donkeys, chimpanzee, cattle, goats, pigs, mice, rats, hamsters, dolphin, foxes, camels, and fowl. In this chapter we describe the clinical characteristics of the disease in felines and canines as well as laboratory tools for diagnosis, and finally address some considerations for public health.

Keywords Feline sporotrichosis • Canine sporotrichosis • Diagnosis • Zoonotic transmission

6.1 Introduction

Sporotrichosis is an implantation mycosis that occurs worldwide and is caused by pathogenic thermodimorphic fungi in the genus *Sporothrix*. The *Sporothrix schenckii sensu lato* clade includes the pathogenic species S. *schenckii sensu stricto*, *S. brasiliensis*, *S. globosa*, and *S. luriei*. *Sporothrix mexicana* and *S. pallida* were described as etiologic agents of sporotrichosis, but both species are placed at a relatively large distance from the clinical clade by phylogenetic analysis, and infections by these species are exceptional (Rodrigues et al. 2014).

The disease has already been reported in humans and several animal species, including cats, dogs, armadillos, horses, mules, donkeys, chimpanzee, cattle, goats, pigs, mice, rats, hamsters, dolphin, foxes, camels, and fowl (Schubach et al. 2012). The first reported cases of natural animal infection were described in Brazil by Lutz and Splendore (1907) in rats. Of all animal species, sporotrichosis has been reported

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most frequently in the cat (Pereira et al. 2014), and the dog is the second most commonly reported animal species affected with infection by *Sporothrix*.

Despite being described on five continents, human sporotrichosis has a higher prevalence in tropical and temperate zones (Barros et al. 2011). At present, the most endemic regions are some areas in Latin America, mainly Brazil, and China. Less frequently, human sporotrichosis occurs in the USA, Japan, Australia, India, and Malaysia; it is rare in Europe.

Animal cases of sporotrichosis were rare until the 1990s, and most of the reports were from the USA (Singer and Muncie 1952; Dunstan et al. 1986; Davies and Troy 1996) and Brazil (Freitas et al. 1965; Larsson et al. 1989). In the past 2 decades, an increased number of feline and canine cases have been described, mainly in the southeast and south regions of Brazil (Nobre et al. 2001; Schubach et al. 2004, 2006; Madrid et al. 2012; Pereira et al. 2010, 2014).

Infection generally occurs via traumatic inoculation of soil, plants, and organic matter contaminated with the filamentous form of the fungus into skin or mucosa. Certain leisure and occupational activities, such as floriculture, agriculture, mining, and wood exploitation, are traditionally associated with this mycosis (Barros et al. 2011).

Zoonotic transmission of sporotrichosis has been described in isolated cases or in outbreaks, primarily in Brazil (Barros et al. 2011), the USA (Ress and Swartzberg 2011), Malaysia (Zamri-Saad et al. 1990), India (Yegneswaran et al. 2009), and Uruguay (Conti Diaz 1989). This mycosis was sporadically associated with scratches or bites from animals such as rodents and dogs and with insect stings (Kauffman 1999). However, the animals most commonly reported as causing zoonotic transmission of *Sporothrix* are cats and armadillos (Conti Diaz 1989; Barros et al. 2004).

Singer and Muncie (1952) described the first case of zoonotic transmission with involvement of sick cats and their owner. However, the role of cats in the transmission of *Sporothrix* has gained attention since the 1980s, when Read and Sperling (1982) reported an outbreak involving five people exposed to a sick cat. Since then, successive reports from different geographical regions have characterized a new risk group for acquisition of sporotrichosis, comprising cat owners and veterinarians. Cats are a proven source of infection, demonstrated by the isolation of the fungus from cutaneous lesions, which usually presents a high fungal burden, and from nails and the nasal and oral cavities of cats with sporotrichosis (Schubach et al. 2012). In the case of armadillos, the animals are not infected but, while trying to escape capture, inflict scratches and thereby inoculate the fungus (Kauffman 1999). *Sporothirx schenckii s. str.* was isolated from soil in an armadillo's burrow in the state of São Paulo, Brazil, supporting that this animal may play a role in the biological cycle of this pathogen and its eco-epidemiology (Rodrigues et al. 2014).

Dogs are probably not directly involved in the zoonotic transmission of *Sporothrix* given the scarcity of viable fungal organisms in their cutaneous lesions and the absence of fungus in the oral cavity. In addition, there were no reports of human cases associated with transmission from dogs in the epidemic in Rio de Janeiro (Barros et al. 2004). However, as with other infectious diseases, dogs with sporotrichosis should be handled with care since their potential public health risk has not been determined (Schubach et al. 2006).

Epidemics are rare and, when they occur, are commonly related to a single source of infection (Bustamante and Campos 2001). The largest outbreak of non-zoonotic sporotrichosis occurred in Witwatersrand, South Africa, where more than 3000 gold miners were infected by the fungus, which was present in the timber of these mines (Helm and Berman 1947). In the USA, the largest epidemic took place in 1988 and involved a total of 84 cases in 15 states, affecting workers who participated in reforestation programs. The cases were associated with exposure to sphagnum moss used for the packing of seedlings from a nursery in Pennsylvania (Dixon et al. 1991).

A retrospective study of 457 human cases of sporotrichosis from 2007 to 2009 indicated a sporotrichosis endemic situation in Jilin province, northeast China. Most of the patients were inhabitants of rural areas where *S. schenckii* had been isolated from the soil and vegetative materials like reeds and corn stalks (Song et al. 2013). Forty-two isolates from these patients were characterized as *S. globosa* according to their phenotypic characteristics and calmodulin gene sequences analysis (Liu et al. 2014).

Zoonotic sporotrichosis is presently occurring as an emerging disease in Brazil, and transmission of *Sporothrix* by sick cats has been increasingly reported. Since 1998, more than 4000 humans (Silva et al. 2012a) and 4000 cats (Gremião et al. 2015) were diagnosed at Fundação Oswaldo Cruz, Rio de Janeiro, emphasizing the importance of this mycosis as a public health problem (Pereira et al. 2014).

In the Brazilian sporotrichosis epidemic, the most prevalent etiological agent is *S. brasiliensis* (Rodrigues et al. 2013), and transmission usually occurs through scratches, bites or contact with sick cats (Barros et al. 2004). Close contact with cats, either with clinically evident disease or without clinical signs, was reported in 91 % of the human cases. Of these, 68 % reported bites and/or scratches (Freitas et al. 2010). In human patients who denied experiencing trauma, unperceived injuries may have easily occurred, especially during animal handling. It is also part of cats' behavior to rub their faces against their handlers, to bite, and to scratch (Barros et al. 2004).

In cats, infection may occur both through traumatic inoculation and/or through inhalation routes. The latter is the likely explanation for the high frequency of respiratory signs, nasal mucosal lesions, and isolation of the fungus from nasal cavity (Schubach et al. 2004), lungs (Schubach et al. 2003b), and bronchoalveolar lavage washings (Leme et al. 2007).

Schubach et al. (2004) had described that more than 90 % of cats became infected after fights and/or contact with other sick cats. Different from the classical route of infection, in which soil and plant material with saprophytic hyphae of the fungus were the source of contamination, transmission of *Sporothrix* by cats to other cats via direct inoculation of yeast cells, which are more virulent than the mycelial form, represents an alternative and successful type of dispersal of the disease (Rodrigues et al. 2013).

In dogs, the infection can be acquired during hunting activities, with possible introduction of the *Sporothrix* through thorn injuries or wood splinters (Rosser and Dunstan 2006). However, in Brazil, the most frequent form of transmission to dogs

has been through scratches from cats, and approximately 80 % of dogs with sporotrichosis had close contact with sick cats (Schubach et al. 2006).

In Brazil, a combination of a highly virulent etiological agent and a susceptible host coupled with low sanitary conditions in the suburbs has made the state of Rio de Janeiro a highly endemic area of this mycosis among animals and humans in the last 2 decades (Silva et al. 2012a; Rodrigues et al. 2013).

6.2 Clinical Aspects

Until the late 1990s, cases of canine and feline sporotrichosis were rarely described in the literature, and knowledge of this mycosis in these animals was based on reports of isolated cases or small outbreaks. Since 1998, the number of descriptions in dogs and cats has increased, especially in Brazil, due to the epizootic that occurs in some states. The significant increase in scientific production on animal sporotrichosis in recent years has provided a broader knowledge of the different aspects of this disease in these species.

Clinical presentations of sporotrichosis may vary according to the immunological status of the host, the load and depth of the inoculum, and the pathogenicity and thermal tolerance of the species, among other factors (Arrillaga-Moncrieff et al. 2009).

The classification of clinical presentation used for humans, according to the location of the lesions, includes cutaneous (lymphocutaneous, fixed, disseminated, or multiple), mucosal, and extracutaneous forms (Barros et al. 2011).

The fixed form is represented by a single lesion or a few lesions at the inoculation site, which is often ulcerated with erythematous edges, without lymphatic involvement (Barros et al. 2011).

The lymphocutaneous form is the most frequent (Lopes-Bezerra et al. 2006). The primary lesion is usually located on the extremities, corresponding to the sites most exposed to trauma. Secondary lesions arise along the path of regional lymphatics, featuring the "sporotrichoid aspect" of the infection (Barros et al. 2011).

A disseminated cutaneous form is characterized by multiple skin lesions at noncontiguous sites without extracutaneous involvement. Until the emergence of the zoonotic transmission, this form was rare and was caused by hematogenous spread of the fungus, usually associated with immunosuppression (Carvalho et al. 2002; Stalkup et al. 2002).

Some authors consider the mucosal form, which preferentially affects the ocular mucosa, to be a variant of the cutaneous form. In the conjunctiva, the granulomatous lesion is accompanied by a serous-purulent discharge, redness, and presence or not of lid edema. In the nasal mucosa, the lesions often involve the septum, with drainage of bloody secretions and detachment of crusts (Schubach et al. 2005).

Among the extracutaneous forms, osteoarticular and pulmonary involvement are the most common (Lopes-Bezerra et al. 2006).

Despite the similarities in clinical presentations observed between humans and animals, the classification described above for the human disease is difficult to transpose to sporotrichosis in dogs and cats. This difficulty is due to the high frequency of fungemia in cats, which is generally associated with the presence of multiple cutaneous lesions with frequent mucosal involvement and extracutaneous signs (Schubach et al. 2004). Additionally, in many instances, an animal may have more than one clinical form simultaneously (Rosser and Dunstan 2006).

The incubation period for *Sporothrix* infection is variable. In humans, the average incubation period is 14 days, with a range of 3–30 days, but it may extend for months (Barros et al. 2013), similar to that observed in animals (Werner and Werner 1994).

6.2.1 *Feline Sporotrichosis*

A higher occurrence of the disease is found in adult male and unneutered cats. In Brazil, the majority of cats are mongrel, and the median age is 2 years (Pereira et al. 2014). The mobility of the cats in the domiciles and surroundings and their involvement in fights, mainly disputes over females, may facilitate the dispersal of *Sporothrix* in the environment and explain the higher prevalence of the disease in adult male and sexually intact animals (Barros et al. 2004).

Feline sporotrichosis has a broad spectrum of disease, ranging from a subclinical infection to multiple skin lesions and fatal disseminated systemic forms. The most frequent clinical forms are multiple skin lesions with mucosal involvement, especially the nasal mucosa (Fig. 6.1). However, skin lesions may not be present in some cases (Schubach et al. 2004). Conjunctival, oral, and genital mucosa may also be affected. In addition, lymphadenitis is frequently observed, different from lymphangitis.

Clinically, skin lesions are characterized particularly by nodules and ulcers and can be found in different anatomical sites, commonly the head, especially nose (Fig. 6.2) (Schubach et al. 2004). Plaques and tumor-like lesions are also observed. The nodules may ulcerate, drain serosanguinolent and/or purulent exudates, and form crusted lesions. Extensive zones of necrosis that expose muscle and bone (Rosser and Dunstan 2006) and myiasis can also occur. Cats with multiple skin and mucosal lesions may exhibit good general health (Schubach et al. 2004).

From the feline sporotrichosis cases seen at Fiocruz and that are part of the epizootic that has been occurring in Rio de Janeiro, the median time between the appearance of cutaneous lesions and the initial clinical visit was 8 weeks (Pereira et al. 2014). Unlike the lesions observed in humans and dogs, cutaneous lesions in felines carry a high fungal burden, making the cat an important source of infection in the zoonotic transmission of this fungus (Werner and Werner 1994).

Extracutaneous signs, particularly respiratory signs (sneezing, dyspnea, and nasal discharge) and nasal mucosa involvement are frequent. Respiratory signs are observed in 41.5 % of the cats, mainly sneezing (39.3 %), which is usually

Fig. 6.1 Feline sporotrichosis. (**a**) Ulcerated skin lesions on the head and right fore- and hind limbs; (**b**) Ulcerated lesions on prepuce and scrotum, exposing the testicles; (**c**) Lymphangitis showing ulcerated skin lesions along the path of regional lymphatics of the right forelimb; (**d**) Ulcerated skin lesion on the metacarpal region; (**e**) Paronychia; (**f**) Tumor-like lesion with ulcers on the nose; (**g**) Multiple crusted lesions on the cephalic region and pinnas; (**h**) Paw necrosis

associated with treatment failure and death (Pereira et al. 2010). The isolation of *Sporothrix* from a nasal swab specimen and the occurrence of sneezing may precede the appearance of skin lesions in some cases (Gremião et al. 2015).

Fig. 6.2 Feline sporotrichosis. (**a**) Nodular lesions on the skin of the nose and mucosal ulceration on the left nasal cavity accompanied by a bilateral serous purulent discharge; (**b**) Multiple ulcerated and crusty skin lesions with conjunctival and nasal mucosal involvement with drainage of serosanguinolent exudate; (**c**) Conjunctivitis showing hyperemic lesion and edema on the lower right palpebral conjunctiva and cutaneous lesions on the head and nose; (**d**) Ulcer on the nasal planum and mucosa

Generally, hematologic and serum biochemical abnormalities are consistent with infectious diseases, and are more frequent in cats presenting multiple skin lesions (Schubach et al. 2004).

Disseminated disease is considered likely if the cat presents with a history of lethargy, depression, anorexia, and fever (Rosser and Dunstan 2006). Hematogenous spread may be an early event and it was demonstrated in vivo by the isolation of the fungus from peripheral blood of cats with widespread cutaneous lesions and from cats with localized skin lesions (Schubach et al. 2003a). Moreover, reports of postmortem examinations performed on naturally infected cats have mentioned the detection of the fungus by histopathological examination and/or by culture in the lungs, heart, spleen, kidneys, lymph nodes, adrenal glands, and liver (Werner and Werner 1994; Dunstan et al. 1986; Schubach et al. 2003b).

The occurrence of severe sporotrichosis in cats is frequently described, even in animals that are apparently immunocompetent, and the dissemination of *Sporothrix* through the bloodstream is unrelated to Feline Immunodeficiency Virus (FIV) and/or the Feline Leukemia Virus (FeLV) co-infection (Barbee et al. 1977; Schubach et al. 2004; Pereira et al. 2010). Several former studies tried to find a

correlation between this particular susceptibility of cats to sporotrichosis and the co-infection with those feline retrovirus, but no association has been detected so far (Schubach et al. 2003a; Pereira et al. 2010; Miranda 2013). Moreover, significant clinical and laboratorial differences were not found among FIV and/or FeLV co-infected and non co-infected animals (Schubach et al. 2004; Pereira et al. 2010).

Little is known so far concerning immunological aspects in feline sporotrichosis. However, the most commonly seen histopathological profile in feline sporotrichosis (Miranda et al. 2013) is consistent with compromised cellular response: large amount of intact yeast cells within macrophages that do not differentiate into epithelioid cells, associated with sparse lymphoplasmacytic infiltrate, leading to granulomas apparently ineffective against *Sporothrix*.

Furthermore, a recent study (Miranda 2013) points toward an important role of CD4+ cells and the CD4:CD8 balance. The cytofluorimetric analysis of lymphocyte subsets in cats with sporotrichosis showed that different types of response may occur and lead to different clinical courses. Some infected cats may present increased levels of CD4+ cells associated with localized lesions, good general condition, well-organized inflammatory response, and a lower fungal load. However, in most cats, an expansion of the CD8+ cells levels is observed due to a pronounced $CD8^{low}$ expression and is associated with widespread lesions and high fungal load. These findings seem to reflect an inverse relationship between increased levels of $CD8^{low}$ cells and infection control, suggesting that this pattern is correlated with high susceptibility.

The relationship between $CD8^{low}$ and the worsening of infections has also been observed in humans (Kundu and Merigan 1991) and may be correlated with type 2 immunity (Kienzle et al. 2002, 2005). Then, the increased levels of the $CD8^{low}$ population could down-regulate cellular immunity (Type 1), which is described as the main protective tool for controlling *Sporothrix* infection (Carlos et al. 1994; Tachibana et al. 1999; Uenotsuchi et al. 2006). This could explain why inefficient granulomas are formed and, thus, how the more severe forms of feline sporotrichosis may develop.

The differential diagnosis includes neoplasia (mainly squamous cell carcinoma), eosinophilic dermatosis, bacterial pyoderma, mycobacteriosis, nocardiosis, cryptococcosis, histoplasmosis, phaeohyphomycosis, and American tegumentary leishmaniasis, among others. Veterinarians should be alert to the possibility of coexisting sporotrichosis and other skin diseases, like neoplasia, eosinophilic dermatosis, bacterial pyoderma, dermatophytosis, and demodicosis.

6.2.2 Canine Sporotrichosis

Sporotrichosis is an uncommon disease in dogs, and present knowledge is derived from a few reports of isolated cases. The largest series of cases involving dogs consisted of 44 animals from Rio de Janeiro, Brazil (Schubach et al. 2006).

The high number of dogs with sporotrichosis observed in the epidemic that occurs in Rio de Janeiro since 1998 is attributed to cats acting as the main source of infection of *Sporothrix* (Barros et al. 2004; Schubach et al. 2004). Interestingly, sporotrichosis in cats preceded its occurrence among their owners and dogs with which they had contact (Schubach et al. 2006).

Different from cats, in which the disease is usually severe, often systemic and difficult to treat, in dogs it has a favorable prognosis (Schubach et al. 2006).

Most reported cases involving dogs have presented the cutaneous form, which is characterized by lesions on the head, ears, neck, back, and thorax. Cutaneous lesions may be not present in some cases. Rare forms of sporotrichosis that can develop in dogs include osteoarticular and disseminated forms (Fig. 6.3a) (Sykes et al. 2001; Crothers et al. 2009). Schubach et al. (2006) described that the majority of dogs with sporotrichosis had good general health and single ulcerated skin lesions on the nose (Fig. 6.3b). According to the same authors, the high frequency of lesions on the muzzle of dogs may be explained by injuries caused by cats that inhabit the same household environments.

The most common lesions in dogs are skin nodules and ulcers, with frequent nasal mucosal involvement, and nasal masses protruding from the nostrils can be observed. Lesions usually are not painful or pruritic. Extracutaneous signs, mainly respiratory, are similar to those described in cats. In addition, most cases had presented lymphadenitis, while lymphangitis was rare (Schubach et al. 2006; Crothers et al. 2009).

In dogs, the hematological and biochemical alterations observed were nonspecific and consistent with infectious diseases, similar to observations made in cats with sporotrichosis (Schubach et al. 2004).

Clinical differentiation of canine sporotrichosis from other conditions like neoplasias, cryptococcosis, histoplasmosis, blastomycosis, bacterial pyoderma, and American tegumentary leishmaniasis must be determined.

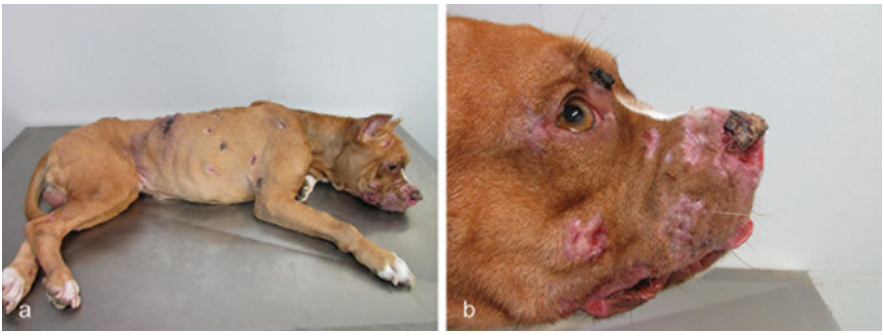

Fig. 6.3 Canine sporotrichosis. (**a**) Multiple ulcerated and crusty lesions on the thorax, hind limbs, flank, and head; (**b**) Detailed view of the skin lesions on the head and nose, showing nasal mucosal involvement

6.3 Laboratorial Diagnosis

The diagnosis of sporotrichosis in dogs and cats depends on laboratorial exams, because clinical signs and gross findings in the necropsy such as ulcers on the skin, mucosa, and enlargement of lymph nodes are nonspecific. The definitive diagnosis of feline sporotrichosis is via fungal isolation in mycological culture (Schubach et al. 2012). However, cytopathology and histopathology are very useful tools for the routine and preliminary diagnosis of this disease, especially in cats due to their high sensitivities in this animal species. The high sensitivities of histopathology and cytopathology for the diagnosis of *Sporothrix* infection in cats are explained by the fact that 54 % of cats with sporotrichosis present high fungal loads in the skin lesions (Miranda et al. 2013). Immunohistochemistry, serology, and polymerase chain reaction (PCR) are other options for the diagnosis of sporotrichosis, but they are used in research and have not yet been routinely implemented. Previous treatment with topical or systemic antifungal drugs in cats and dogs can reduce the sensitivity of laboratorial exams for the diagnosis of *Sporothrix* infection in these animals.

6.3.1 Cytopathology

This test is very useful due to its rapidity, simplicity, and low cost. In addition, the sensitivity of this method ranges from 79 to 85 % in samples collected from skin lesions of cats with sporotrichosis (Pereira et al. 2011; Silva et al. 2015). However, a sensitivity of 32 % was observed in dogs (Santos et al. 2007). This lower sensitivity of cytopathology for the diagnosis of sporotrichosis in dogs is related to the frequent small number of yeast-like structures of *Sporothrix* in the skin lesions of these animals (Welsh 2003; Santos et al. 2007). Suitable samples for cytopathology are impression smears on glass slides for microscopy of exudates of ulcerated skin, smears of swab specimens, tissue samples from biopsy or necropsy, skin scrapings, and also aspirates of abscess and nodules. The smears are stained with Wright's or Romanowsky-type (e.g., Wright, DiffQuik or Quick Panoptic) or Grocott's methenamine silver (GMS). Positive cytopathological exam stained by Romanowsky-type staining reveals cigar-shaped to oval or round budding yeast-like organisms of 3–5 μm by 5–9 μm, with blue cytoplasm and a single, pink nucleus surrounded by nonstaining cell wall within macrophages, neutrophils, or extracellular (Fig. 6.4) (Clinkenbeard 1991; Pereira et al. 2011).

6.3.2 Histopathology

This technique is important because it allows not only the preliminary diagnosis of *Sporothrix* but also the correlation of this fungus with associated lesions in tissues.

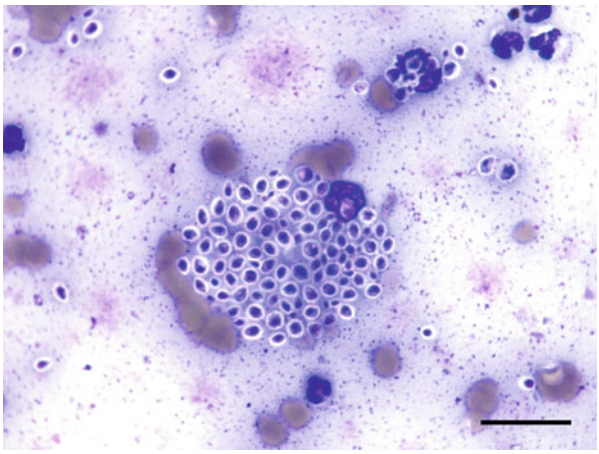

Fig. 6.4 Feline sporotrichosis. Impression smear from ulcerated skin lesion showing numerous cigar-shaped to oval or round budding yeast-like organisms fulfilled with blue cytoplasm with a single round pink nucleus surrounded by a non-staining cell wall within macrophages and extracellular. Quick Panoptic stain. Bar = 0.02 mm

It is also frequently used for semi-quantification of fungal load and for pathogenesis and virulence studies. Tissue samples for histopathology are collected by biopsy or necropsy. The skin biopsies should be obtained from borders of active lesions with a 3–4 mm surgical punch, after local antisepsis with 70 % alcohol and anesthesia with 2 % lidocaine. The collected tissues are fixed in 10 % buffered formalin, embedded in blocks of paraffin wax, microtome sectioned to 5 μm thickness, and stained (Carson and Hladick 2009). The hematoxylin-eosin (H&E) is the routine staining for observation of the microscopic alterations associated with *Sporothrix* infection. However, H&E staining does not enable good visualization of the morphology of yeast-like forms of *Sporothrix*, which can be confused with other pathogenic fungi and protozoa. Moreover, the microscopic alterations observed are not disease specific (Schubach et al. 2012). Therefore, specific histochemical staining such as GMS and periodic acid-Schiff (PAS) should be used in serial sections of the same tissue sample to increase the sensitivity and specificity of histological diagnosis of *Sporothrix* (Miranda et al. 2009, 2011). Positive histological exam stained by specific histochemical techniques shows cigar-shaped to oval or round yeast-like forms of 4–6 μm in size that generally exhibit a single budding with a narrow base, and are dark stained in GMS (Fig. 6.5) and pink stained in PAS (Fig. 6.6). These yeast-like forms are observed intracellular within neutrophils, macrophages, giant cells, and osteoclasts or extracellular (Gremião et al. 2015). Pseudohyphae, hyphae, and yeast-like forms with multiple budding can also be seen (Werner et al. 1971; Gremião et al. 2015). The main histological differential diagnosis of sporotrichosis is histoplasmosis. The yeast-like forms of *Histoplasma capsulatum* are round to oval and may exhibit a single budding with a narrow base similar to *Sporothrix*. However, there are no cigar-shaped yeast-like forms of *H. capsulatum*. In addition, the round to oval yeast-like forms of this fungus are smaller than those of *Sporothrix*, measuring from 2 to 4 μm, and usually grouped in clusters inside macrophages (Guarner and Brand 2011). The sensitivity of GMS for the diagnosis of yeast-like forms of *Sporothrix* in skin lesions is 94 % in cats and

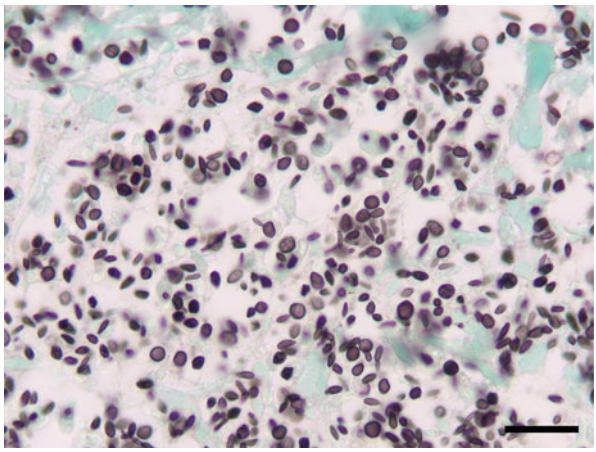

Fig. 6.5 Feline sporotrichosis. Skin. Abundant dark-stained cigar-shaped and round-to-oval yeasts, some of them exhibiting a single budding with a narrow base. GMS. Bar = 0.02 mm

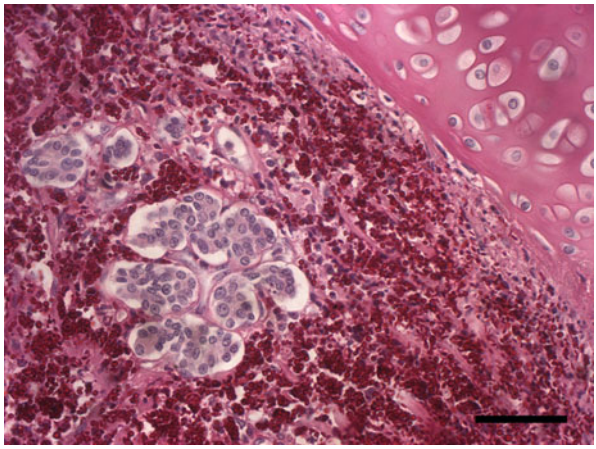

Fig. 6.6 Feline sporotrichosis. Nasal mucosa. Pyogranulomatous rhinitis showing abundant pink-stained cigar-shaped and round-to-oval yeasts, some exhibiting a single budding with a narrow base, within macrophages and extracellular. PAS. Bar = 0.06 mm

44 % in dogs using isolation of *Sporothrix* by mycological culture as the reference standard (Miranda et al. 2011, 2013). The PAS is less sensitive than GMS for the diagnosis of *Sporothrix* in skin lesions of dogs and detects yeast-like forms of this fungus in approximately 19 % of positive cases in mycological culture (Miranda et al. 2011). In cats, the sensitivity of PAS has not been evaluated.

Histologically, the cutaneous lesions of feline and canine sporotrichosis are characterized by ulcerative and variable but usually intense pyogranulomatous inflammatory reactions in the dermis (Fig. 6.7), which can reach the panniculus (Schubach et al. 2004; Miranda et al. 2009, 2013). Unlike dogs and humans, systemic involvement is common in cats, and a pyogranulomatous inflammatory reaction and yeasts can be observed in the lungs, spleen, lymph nodes, liver, kidneys, adrenal glands, eyes (Werner et al. 1971; Dunstan et al. 1986; Schubach

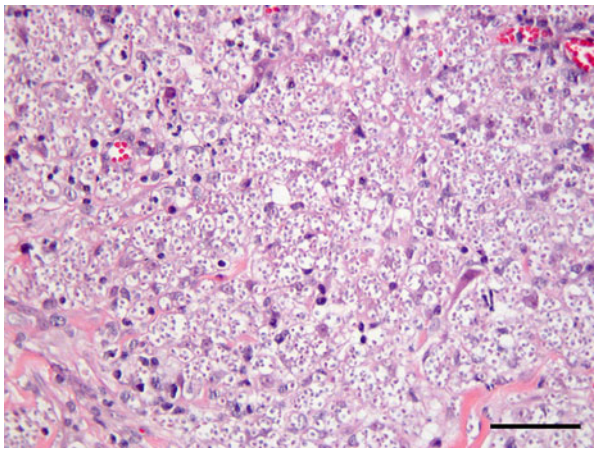

Fig. 6.7 Feline sporotrichosis. Skin. Severe pyogranulomatous dermatitis showing a poorly organized granuloma in which macrophages are filled with yeast-like forms throughout its length; rare lymphocytes and plasma cells. This type of poorly organized granuloma is denominated fungal granuloma.
HE. Bar = 0.05 mm

et al. 2003b), skeletal muscles, and tongue. Additionally, in the noses of cats with sporotrichosis, a high frequency of severe pyogranulomatous rhinitis (Fig. 6.6) with osteomyelitis, lyses of bone, and necrosis of hyaline cartilage of vestibule were associated with the presence of *Sporothrix* organisms (Gremião et al. 2015). These microscopic alterations help to explain why lesions on the nose are difficult to treat in cats with sporotrichosis. There is an inverse correlation between well-formed granulomas and number of yeasts in dogs and cats (Schubach et al. 2004; Miranda et al. 2009, 2013). In cats with sporotrichosis, the frequency of well-formed granulomas in the skin lesions is 11 %, which is much lower than that observed for dogs (38–45 %) and humans (38 %) (Schubach et al. 2006; Miranda et al. 2009, 2013; Quintella et al. 2011). Also, poorly organized granulomas in which macrophages are filled with yeast-like forms throughout the length of the granuloma, and rarely lymphocytes and plasma cells are observed, were reported only in the skin of cats with sporotrichosis. This type of poorly organized granuloma was denominated fungal granuloma (Fig. 6.7) and was observed in the skin lesions of 40 % of the cats with sporotrichosis from the endemic region of Rio de Janeiro, Brazil (Miranda et al. 2013). The low frequency of well-formed granulomas and the great quantity of yeasts in the lesions reveal the higher susceptibility of cats to sporotrichosis compared with humans and dogs (Schubach et al. 2004; Miranda et al. 2009, 2013; Quintella et al. 2011). Multinucleated giant cells are infrequent in dogs and cats compared with their occurrence in humans (Miranda et al. 2009, 2013; Quintella et al. 2011). Asteroid bodies have not been reported in dogs and cats (Schubach et al. 2004, 2012; Miranda et al. 2009).

6.3.3 Immunohistochemistry

This technique can be applied to improve the sensitivity of histological diagnosis of sporotrichosis in formalin-fixed paraffin-embedded tissues of dogs. Its sensitivity is

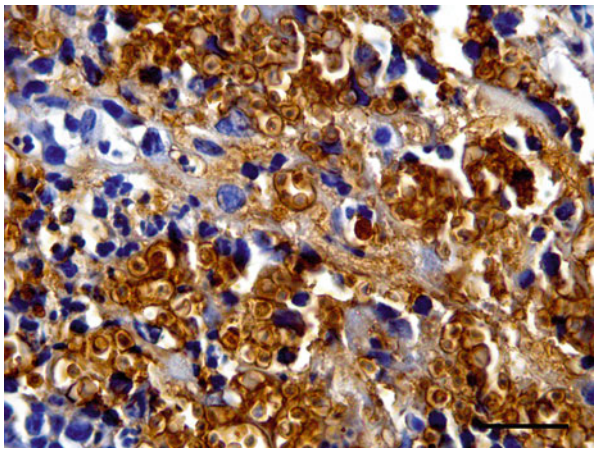

Fig. 6.8 Feline sporotrichosis. Skin. Abundant dark-brown stained cigar-shaped, round-to-oval yeasts within macrophages and extracellular. Immunohistochemistry. Bar = 0.01 mm

65 %, higher than GMA and PAS (Miranda et al. 2011). In cats, the sensitivity of immunohistochemistry has not been evaluated for the diagnosis of sporotrichosis. According to Miranda et al. (2011), deparaffinized tissue sections of 5 μm are incubated with a rabbit anti-*Sporothrix* polyclonal serum at a dilution of 1:4000. For the detection step, the sections are incubated with universal biotinylated secondary antibody and streptavidin–peroxidase complex. The reaction is developed using diaminobenzidine as chromogen, and yeast-like forms of *Sporothrix* are stained dark brown (Fig. 6.8).

One disadvantage of this method is that no anti-*Sporothrix* antibodies are commercially available. Therefore, rabbit anti-*Sporothrix* polyclonal serum is produced in-house via the immunization of rabbits inoculated intravenously with four doses of soluble protein extracts of mycelia of *Sporothrix* (Lopes-Alves et al. 1994).

6.3.4 Mycological Culture

The fungal isolation in mycological culture is the reference method for the diagnosis of sporotrichosis in animals and humans due to its high sensitivity and specificity. In addition, the identification of *Sporothrix* at species level depends on mycological culture for morphological and physiological phenotyping of the fungus, and provision of fungal isolates for PCR (Marimon et al. 2007; Rodrigues et al. 2013). Clinical samples for the diagnosis of sporotrichosis in dogs and cats by mycological culture include swab specimens of the nasal cavities, exudative lesions, purulent or seropurulent content aspirated from nonulcerated abscesses, and surgical skin biopsy specimens (Schubach et al. 2004; Santos et al. 2007). In cats, high sensitivity of mycological culture for the diagnosis of *Sporothrix* infection is obtained via both skin biopsy and swab specimens of exudative lesions, but

the latter is preferable because it is less invasive. Nonetheless, the most indicated sample for the mycological diagnosis of *Sporothrix* infection in dogs is surgical skin biopsy, because it shows the best results when compared with the swab samples (Schubach et al. 2004, 2006). Additionally, in case of suspicion of the disseminated form of sporotrichosis in cats, 3 ml of whole blood can be collected to diagnose hematogenous spread of *Sporothrix* spp. (Schubach et al. 2003a). The swabs should be seeded on Sabouraud dextrose agar with chloramphenicol and Sabouraud dextrose agar with chloramphenicol plus cycloheximide (Mycosel agar) and then sent to laboratory at room temperature. The skin biopsies should be obtained from borders of active lesions in the same way as described for collecting skin samples for histopathology. The tissue samples should be kept in a sterile glass flask with sterile saline and antibacterial agent and transported at refrigerated temperatures (4 °C) to a diagnostic laboratory. The whole blood can be directly seeded in blood culture flasks and sent to laboratory at room temperature. For a good sensitivity of mycological culture, the clinical samples should be sent as soon as possible to the laboratory, preferentially within 24 h.

In the laboratory, the clinical samples should be inoculated onto Sabouraud agar and Mycosel agar, incubated at 25–30 °C, and observed for 4 weeks (Pappas et al. 2000; Barros et al. 2011). Mycelial growth of *Sporothrix* spp. is usually observed in 5–7 days (Barros et al. 2011). Initially, typical colonies are cream-colored, smooth and moist, gradually becoming dark brown or black, usually in the centers of the colonies. For blood culture, flasks with 3 ml of whole blood are incubated at room temperature in an inverted position. One milliliter of sediment is aspirated on the 2nd and 7th days and inoculated into flasks containing agar-brain-heart-infusion (BHI) medium, incubated at 25 °C, and observed for 6 weeks for fungal growth (Schubach et al. 2003a). Suspected isolates are subcultivated onto potato-dextrose agar at 25 °C. Microscopically, the mycelia form of *Sporothrix* spp. produces thin septate hyphae, 1–2 μm in diameter, solitary, erect and tapered toward the apex. Conidiophores arise at right angles from the thin septate hyphae, and conidia are formed in clusters on tiny denticles by sympodial proliferation of the conidiophores with arrangement suggestive of a flower. Conidia are obovoidal, hyaline, one celled and smooth-walled, measuring 1.5–2.5 × 2.5–5.5 μm. Conidia sessile are hyaline or darkly pigmented, thick walled, one celled, and globose/subglobose, connected individually throughout the hyphae. These conidia measure from 2.5 to 4.0 μm in diameter (Lavalle and Mariat 1983; de Hoog et al. 2004).

In addition to morphology of their mycelial form, the identification of the fungus isolate as *Sporothrix* spp. depends on its thermal dimorphism property. The thermal dimorphism of *Sporothrix* is demonstrated by the conversion of its mycelial form to the yeast form by subculturing the fungus on enriched media such as BHI, chocolate agar, and blood agar at 37 °C for 5–7 days (Barros et al. 2011). After *Sporothrix* conversion to the yeast phase, colonies acquire a creamy aspect and a yellow to tan color (Morris-Jones 2002). Microscopically, the yeast form is oval- or cigar-shaped and exhibit single or multiple buds with a narrow base.

The drawbacks of culture are that it is time consuming, taking from 10 to 15 days to complete, and false negatives can occur due to low fungal burden, prior treatment

with topical or systemic antifungal agents, inadequate sample, and excessive time to ship the samples to laboratory. Also, this method is susceptible to contamination with microorganisms such as bacteria and mites.

6.3.5 PCR

PCR is a rapid and precise technique that has been used to identify *Sporothrix* at species level in culture samples of cats (Rodrigues et al. 2013), particularly in isolates not identified by morphological and physiological phenotyping (Marimon et al. 2007). Different PCR methodologies targeting different genes are applied for this purpose. PCR sequencing targeting the calmodulin gene is the most used, but PCR sequencing targeting ITS1, β-tubulin, chitin synthase, large subunit, and PCR fingerprinting using the universal primer T3B also have good results to distinguish among species of the *Sporothrix* complex (Oliveira et al. 2011, 2014; Rodrigues et al. 2013, 2014; Zhou et al. 2014). For clinical samples, a PCR targeting the chitin synthase 1 (CHS 1) was used with success for specific and rapid detection of *Sporothrix* DNA in a biopsy specimen of a cat with sporotrichosis (Kano et al. 2005). However, the sensitivity and specificity of this PCR and other PCR methodologies recently described have not been evaluated in clinical samples of dogs and cats for the diagnosis of *Sporothrix* at species level.

6.3.6 Serology

The enzyme-linked immunosorbent assay may be used as a sensitive and specific screening tool for the detection of *Sporothrix* antibodies in the serum of cats infected by this fungus (Fernandes et al. 2011). The sensitivity of this test was 96 % and specificity was 98 % using *S. schenckii* crude exoantigen. Additionally, this test is inexpensive, quick to perform, and easy to interpret; however, it is not available commercially.

6.4 Conclusions and Future Perspectives

Feline sporotrichosis presents a significant zoonotic potential, but dogs are probably not directly involved in the zoonotic transmission of *Sporothrix*. Sporotrichosis in cats requires preventive measures to avoid transmission within the species and from animals to humans (Barros et al. 2011).

Owners of infected cats should be alerted to the possibility of infection. Care must be taken to avoid penetration injuries and contact with exudate from lesions when handling cats with sporotrichosis (Taboada 2000; Schubach et al. 2012).

Specific biosafety procedures to reduce risk during the handling of cats with potential sporotrichosis, such as using personal protective equipment, should be followed by veterinarians, technicians, caretakers, and owners of sick cats (Silva et al. 2012b). The use of disposable latex gloves is compulsory. After the careful removal of gloves, the hands, wrists, and forearms should be washed with chlorhexidine or povidone-iodine (Rosser and Dunstan 2006). When cutaneous lesions are caused by scratches or bites from cats with sporotrichosis, it is recommended they be washed immediately with soap or the antiseptics cited above, and medical assistance sought.

Preventive measures, such as early spaying, must be used for cats (Pereira et al. 2014). Cats with sporotrichosis should be correctly treated and kept isolated in a suitable place. In the case of cats with no possibility of treatment, euthanasia and cremation should be standard procedures in a veterinary health center (Barros et al. 2011).

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References

Arrillaga-Moncrieff I, Capilla J, Mayayo E et al (2009) Different virulence levels of the species of *Sporothrix* in a murine model. Clin Microbiol Infect 15(7):651–655

Barbee WC, Ewert A, Davidson EM (1977) Animal model of human disease: sporotrichosis. Am J Pathol 86(1):281–284

Barros MB, Schubach AO, Do Valle AC et al (2004) Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: description of a series of cases. Clin Infect Dis 38(4):529–535

Barros MB, Almeida Paes R, Schubach AO (2011) *Sporothrix schenckii* and sporotrichosis. Clin Microbiol Rev 24(4):633–654

Barros MB, Pereira SA, Schubach AO et al (2013) Esporotricose. In: Coura JR (ed) Dinâmica das doenças infecciosas e parasitárias, 2nd edn. Guanabara Koogan, Rio de Janeiro, pp 1196–1206

Bustamante B, Campos PE (2001) Endemic sporotrichosis. Curr Opin Infect Dis 14(2):145–149

Carlos IZ, Zini MM, Sgarbi DB et al (1994) Disturbances in the production of interleukin-1 and tumor necrosis factor in disseminated murine sporotrichosis. Mycopathologia 127(3):189–194

Carson FL, Hladick C (2009) Histotechnology: a self instructional text, 3rd edn. ASCP Press, Chicago

Carvalho MT, Castro AP, Baby C et al (2002) Disseminated cutaneous sporotrichosis in a patient with AIDS: report of a case. Rev Soc Bras Med Trop 35(6):655–659

Clinkenbeard KD (1991) Diagnostic cytology: sporotrichosis. Compend Contin Educ Pract Vet 13(2):207–211

Conti Diaz IA (1989) Epidemiology of sporotrichosis in Latin America. Mycopathologia 108(2):113–116

Crothers SL, White SD, Ihrke PJ et al (2009) Sporotrichosis: a retrospective evaluation of 23 cases seen in northern California (1987-2007). Vet Dermatol 20(4):249–259. doi:10.1111/j.1365-3164.2009.00763.x

Davies C, Troy GC (1996) Deep mycotic infections in cats. J Am Anim Hosp Assoc 32(5): 380–391, http://dx.doi.org/10.5326/15473317-32-5-380

De Hoog GS, Guarro J, Gené J et al (2004) Atlas of clinical fungi. CD Version, edited by T. Weniger, Computer Science II, University of Wurzburg, Würzburg, Germany

Dixon DM, Salkin IF, Duncan RA et al (1991) Isolation and characterization of *Sporothrix schenckii* from clinical and environmental sources associated with the largest U.S. epidemic of sporotrichosis. J Clin Microbiol 29(6):1106–13

Dunstan RW, Langham RF, Reinmann KA et al (1986) Feline sporotrichosis: a report of five cases with transmission to humans. J Am Acad Dermatol 15(1):37–45

Fernandes GF, Lopes-Bezerra LM, Bernardes-Engemann AR et al (2011) Serodiagnosis of sporotrichosis infection in cats by enzyme-linked immunosorbent assay using a specific antigen, SsCBF, and crude exoantigens. Vet Microbiol 147(3-4):445–449. doi:10.1016/j.vetmic.2010.07.007

Freitas D, Moreno G, Saliba A et al (1965) Esporotricose em cães e gatos. Rev Fac Med Vet Univ Sao Paulo 7(2):381–387, http://dx.doi.org/10.11606/issn.2318-5066.v7i2p381-388

Freitas DF, Valle AC, Almeida Paes R et al (2010) Zoonotic sporotrichosis in Rio de Janeiro, Brazil: a protracted epidemic yet to be curbed. Clin Infect Dis 50(3):453

Gremião IDF, Menezes RC, Schubach TMP et al (2015) Feline sporotrichosis: epidemiological and clinical aspects. Med Mycol 53(1):15–21

Guarner J, Brand ME (2011) Histopathologic diagnosis of fungal infections in the 21st century. Clin Microbiol Rev 24(2):247. doi:10.1128/CMR.00053-10

Helm M, Berman C (eds) (1947) The clinical, therapeutic and epidemiological features of sporotrichosis infection of the mines. Proceedings of the Transvaal Mine Medical Officers' Association. Sporotrichosis infection on mines of the Witwatersrand. The Transvaal Chamber of Mines, Johannesburg

Kano R, Watanabe K, Murakami M et al (2005) Molecular diagnosis of feline sporotrichosis. Vet Rec 156(15):484–485. doi:10.1136/vr.156.15.484

Kauffman CA (1999) Sporotrichosis. Clin Infect Dis 29(2):231–236

Kienzle N, Buttigieg K, Groves P et al (2002) A clonal culture system demonstrates that IL-4 induces a subpopulation of noncytolytic T cells with low CD8, perforin, and granzyme expression. J Immunol 168(4):1672–1681. doi:10.4049/jimmunol.168.4.1672

Kienzle N, Olver S, Buttigieg K et al (2005) Progressive differentiation and commitment of CD8+ T cells to a poorly cytolytic CD8low phenotype in the presence of IL-4. J Immunol 174(4):2021–2029. doi:10.4049/jimmunol.174.4.2021

Kundu SK, Merigan TC (1991) Inverse relationship of CD8+ CD11+ suppressor T cells with human immunodeficiency virus (HIV)-specific cellular cytotoxicity and natural killer cell activity in HIV infection. Immunology 74:567–571

Larsson CE, Goncalves MA, Araujo VC et al (1989) Feline sporotrichosis: clinical and zoonotic aspects. Rev Inst Med Trop Sao Paulo 31(5):351–358

Lavalle P, Mariat F (1983) Sporotrichosis. Bull Inst Pasteur 81:295–322

Leme LR, Schubach TM, Santos IB et al (2007) Mycological evaluation of bronchoalveolar lavage in cats with respiratory signs from Rio de Janeiro, Brazil. Mycoses 50(3):210–214

Liu TT, Zhang K, Zhou X (2014) Molecular identification of *Sporothrix* clinical isolates in China. J Zhejiang Univ Sci B 15(1):100–108

Lopes-Alves L, Travassos LR, Previato JO et al (1994) Novel antigenic determinants from peptidorhamnomannans of *Sporothrix schenckii*. Glycobiology 4(3):281–288

Lopes-Bezerra LM, Schubach A, Costa RO (2006) *Sporothrix schenckii* and sporotrichosis. An Acad Bras Cienc 78(2):293–308

Lutz A, Splendore A (1907) Sobre uma mycose observada em homens e ratos. Rev Med Sao Paulo 21:433–450

Madrid IM, Mattei AS, Fernandes CG et al (2012) Epidemiological findings and laboratory evaluation of sporotrichosis: a description of 103 cases in cats and dogs in southern Brazil. Mycopathologia 173(4):265–273

Marimon R, Cano J, Gene J et al (2007) *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol 45(10):3198–3206. doi:10.1128/JCM.00808-07

Miranda LHM (2013) Evaluation of the inflammatory response in vivo and in vitro in feline sporotrichosis at different clinical presentations. Thesis [PhD in Clinical Research in Infectious Diseases] Evandro Chagas Clinical Research Institute

Miranda LHM, Quintella LP, Santos IB et al (2009) Histopathology of canine sporotrichosis: a study of 86 cases from Rio de Janeiro (2001-2007). Mycopathologia 168(2):79–87

Miranda LHM, Quintella LP, Menezes RC et al (2011) Evaluation of immunohistochemistry for the diagnosis of sporotrichosis in dogs. Vet J 190(3):408–411

Miranda LHM, Conceição-Silva F, Quintella LP et al (2013) Feline sporotrichosis: histopathological profile of cutaneous lesions and their correlation with clinical presentation. Comp Immunol Microbiol Infect Dis 36(4):425–432

Morris-Jones R (2002) Sporotrichosis. Clin Exp Dermatol 27(6):427–431

Nobre MO, Castro AP, Caetano D et al (2001) Recurrence of sporotrichosis in cats with zoonotic involvement. Rev Iberoam Micol 18:137–140

Oliveira MME, Almeida-Paes R, Muniz MM et al (2011) Phenotypic and molecular identification of *Sporothrix* isolates from an epidemic area of sporotrichosis in Brazil. Mycopathologia 172(4):257–267

Oliveira MME, Almeida-Paes R, Gutierrez-Galhardo MC et al (2014) Molecular identification of the *Sporothrix schenckii* complex. Rev Iberoam Micol 31(1):2–6

Pappas PG, Tellez I, Deep AE et al (2000) Sporotrichosis in Peru: description of an area of hyperendemicity. Clin Infect Dis 30(1):65–70

Pereira SA, Passos SR, Silva JN et al (2010) Response to azolic antifungal agents for treating feline sporotrichosis. Vet Rec 166(10):290–294

Pereira SA, Menezes RC, Gremião ID et al (2011) Sensitivity of cytopathological examination in the diagnosis of feline sporotrichosis. J Feline Med Surg 13(4):220–223

Pereira SA, Gremião ID, Kitada AA et al (2014) The epidemiological scenario of feline sporotrichosis in Rio de Janeiro, State of Rio de Janeiro, Brazil. Rev Soc Bras Med Trop 47(3):392–393

Quintella LP, Passos SRL, Galhardo ACF et al (2011) Histopathology of cutaneous sporotrichosis in Rio de Janeiro: a series of 119 consecutive cases. J Cut Pathol 38(1):25–32

Read SI, Sperling LC (1982) Feline sporotrichosis: transmission to man. Arch Dermatol 118(6):429–431

Ress RK, Swartzberg JE (2011) Feline-transmitted sporotrichosis: a case study from California. Dermatol Online J 17(6):2

Rodrigues AM, Teixeira MM, de Hoog GS et al (2013) Phylogenetic analysis reveals a high prevalence of *Sporothrix brasiliensis* in feline sporotrichosis outbreaks. PLoS Negl Trop Dis 7(6):e2281

Rodrigues AM, de Hoog GS, Camargo ZP (2014) Genotyping species of the *Sporothrix schenckii* complex by PCR-RFLP of calmodulin. Diagn Microbiol Infect Dis 78(4):383–387

Rosser E, Dunstan R (2006) Sporotrichosis. In: Greene CE (ed) Infectious diseases of the dog and cat, 3rd edn. Saunders Elsevier, Philadelphia

Santos IB, Schubach TM, Leme LR et al (2007) Sporotrichosis: the main differential diagnosis with tegumentary leishmaniosis in dogs from Rio de Janeiro, Brazil. Vet Parasitol 143(1):1–6

Schubach TM, Schubach A, Okamoto T et al (2003a) Haematogenous spread of *Sporothrix schenckii* in cats with naturally acquired sporotrichosis. J Small Anim Pract 44(9):395–398

Schubach TM, Schubach Ade O, Cuzzi-Maya T et al (2003b) Pathology of sporotrichosis in 10 cats in Rio de Janeiro. Vet Rec 152(6):172–175

Schubach TM, Schubach A, Okamoto T et al (2004) Evaluation of an epidemic of sporotrichosis in cats: 347 cases (1998-2001). J Am Vet Med Assoc 224(10):1623–1629

Schubach AO, Schubach TM, Barros MB (2005) Epidemic cat-transmitted sporotrichosis. N Engl J Med 353(11):1185–1186

Schubach TM, Schubach A, Okamoto T et al (2006) Canine sporotrichosis in Rio de Janeiro, Brazil: clinical presentation, laboratory diagnosis and therapeutic response in 44 cases (1998-2003). Med Mycol 44(1):87–92

Schubach TM, Menezes RC, Wanke B (2012) Sporotrichosis. In: Greene CE (ed) Infectious diseases of the dog and cat, 4th edn. Saunders Elsevier, Philadelphia, pp 645–650

Silva MB, Costa MM, Torres CC et al (2012a) Urban sporotrichosis: a neglected epidemic in Rio de Janeiro, Brazil. Cad Saude Publica 28(10):1867–1880

Silva DT, Menezes RC, Gremião ID et al (2012b) Zoonotic sporotrichosis: biosafety procedures. Acta Sci Vet 40(4):1067

Silva JN, Passos SRL, Menezes RC et al (2015) Diagnostic accuracy assessment of cytopathological examination of feline sporotrichosis. Med Mycol. doi: 10.1093/mmy/myv038

Singer JI, Muncie JE (1952) Sporotrichosis; etiologic considerations and report of additional cases from New York. N Y State J Med 52(17):2147–2153

Song Y, Li SS, Zhong SX et al (2013) Report of 457 sporotrichosis cases from Jilin province, northeast China, a serious endemic region. J Eur Acad Dermatol Venereol 27(3):313–318

Stalkup JR, Bell K, Rosen T (2002) Disseminated cutaneous sporotrichosis treated with itraconazole. Cutis 69(5):371–374

Sykes JE, Torres SM, Armstrong PJ et al (2001) Itraconazole for treatment of sporotrichosis in a dog residing on a Christmas tree farm. J Am Vet Med Assoc 218(9):1440–1443, 9

Taboada J (2000) Systemic mycoses. In: Ettinger S, Feldman E (eds) Textbook of veterinary internal medicine - diseases of the dog and cat, 5th edn. W. B. Saunders Company, Philadelphia, pp 453–476

Tachibana T, Matsuyama T, Mitsuyama M (1999) Involvement of CD4+ T cells and macrophages in acquired protection against infection with *Sporothrix schenckii* in mice. Med Mycol 37(6):397–404

Uenotsuchi T, Takeuchi S, Matsuda T et al (2006) Differential induction of Th1-prone immunity by human dendritic cells activated with *Sporothrix schenckii* of cutaneous and visceral origins to determine their different virulence. Int Immunol 18(12):1637–1646

Welsh RD (2003) Sporotrichosis. J Am Vet Med Assoc 223(8):1123–1126

Werner AH, Werner BE (1994) Sporotrichosis in man and animal. Int J Dermatol 33(10):692–700

Werner RE Jr, Levine BG, Kaplan W et al (1971) Sporotrichosis in a cat. J Am Vet Med Assoc 159(4):407–411

Yegneswaran PP, Sripathi H, Bairy I et al (2009) Zoonotic sporotrichosis of lymphocutaneous type in a man acquired from a domesticated feline source: report of a first case in southern Karnataka, India. Int J Dermatol 48(11):1198–1200

Zamri-Saad M, Salmiyah TS, Jasni S et al (1990) Feline sporotrichosis: an increasingly important zoonotic disease in Malaysia. Vet Rec 127(19):480

Zhou X, Rodrigues AM, Feng P et al (2014) Global ITS diversity in the *Sporothrix schenckii* complex. Fungal Divers 66(1):153–165

Chapter 7
Models of Experimental Sporotrichosis and Immune Response Against *Sporothrix schenckii*

Iracilda Zeppone Carlos, Lucas Souza Ferreira, and Amanda Costa Gonçalves

Abstract Antifungal host responses can vary depending on the site of infection, fungal pathogen, fungal morphotype (yeast versus hyphae), and immune status of the host. Variation in the virulence of individual *Sporothrix schenckii* strains and the immune status of the host may both contribute to the variety in the clinical manifestations of sporotrichosis. However, the factors involved in the pathogenesis of sporotrichosis and the mechanisms determining *S. schenckii* virulence remain unclear. Classic murine models of sporotrichosis display a characteristic transitory state of depressed cell-mediated immunity during the disease's acute phase, which has been suggested to result from the nitric oxide–induced T-cell apoptosis and loss of responsiveness to mitogens. In sporotrichosis, recognition of the *S. schenckii* lipid components, through Toll-like receptor (TLR)-4 or via an inflammasome-dependent pathway, seems to drive inflammation, whereas the TLR2-mediated recognition of the fungus's exoantigen may serve as an escape mechanism, although the *S. schenckii* internalization by TLR2$^{-/-}$ macrophages is almost completely abrogated, in vitro at least. Finally, both in vitro and in vivo studies have suggested the adaptive immune response against *S. schenckii* to be of a mixed Th1/Th17 pattern, with a predominance of Th17 and Th1/Th17 mixed cells over Th1 cells. In this chapter we discuss the current understanding of the immune mechanisms triggered by *S. schenckii sensu strictu*, along with the animal models used so far to study this pathogen.

Keywords *Sporothrix schenckii* • Immune response • Animal model • Th17 cells • Cytokines

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7.1 Introduction

Antifungal host responses can vary depending on the site of infection, fungal pathogen, fungal morphotype (yeast versus hyphae), and the host's immune status. Variation in the virulence of individual *Sporothrix schenckii* strains and the immune status of the host may both contribute to the variety in clinical manifestations of sporotrichosis. However, the factors involved in the pathogenesis of sporotrichosis and the mechanisms determining *S. schenckii* virulence remain unclear. In this chapter we discuss the current understanding of the immune mechanisms triggered by *S. schenckii sensu strictu*, along with the animal models used so far to study this pathogen.

7.2 Animal Models of Sporotrichosis

Animal models are critical for determining the mechanisms of induced protection against mycosis (Steele and Wormley 2012). In designing an animal model to study fungal pathogenesis, host antifungal immune responses, and antifungal compounds, the common features of vertebrate animals must be taken into account. Mice and other laboratory animals have proven invaluable in modeling the clinical syndromes associated with the cutaneous, subcutaneous, and life-threatening invasive forms of sporotrichosis, generating experimental models that reproduce the disease's main features (Fig. 7.1).

An experimental model of sporotrichosis has been induced in hamsters by the intraperitoneal (i.p.) inoculation of *S. schenckii*. The disease was characteristically chronic and disseminated with gummy lesions in which fusiform, ovoid, and rounded cells were observed (Lurie 1971). Later, Charoenvit and Taylor (1979)

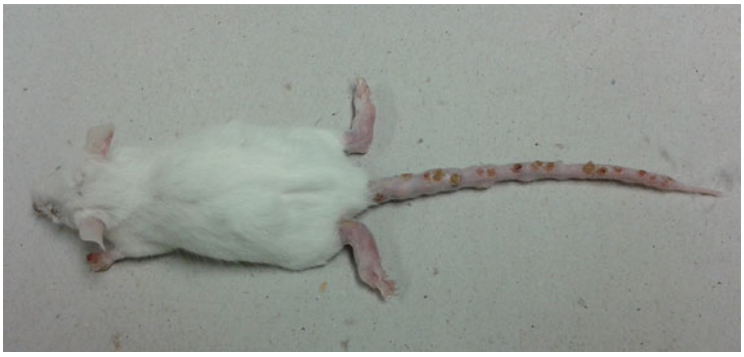

Fig. 7.1 Characteristic aspects of sporotrichosis in a Balb/c mouse inoculated with 10^7 yeast cells of *Sporothrix schenckii* ATCC 16345 through the intraperitoneal route. The photo was taken around 75 days post-inoculation. A series of nodular and/or ulcerated lesions following the course of the lymphatic vessels can be seen on the animal's tail and paws

simulated the lymphatic disease, similar to that observed in humans, in hamsters by the cutaneous inoculation of the fungus in the animals' paw. The infection produced by 5.3×10^3 *S. schenckii* yeast cells was limited to the lymphatic vessels, whereas an inoculum 10^3-fold greater led to a systemic nonfatal disease involving the liver and spleen.

Although other animals have been used, the murine model is one of the most studied in sporotrichosis. Kazanas (1986) evaluated the influence of age and route of infection in the frequency of sporotrichosis in Swiss mice. Animal susceptibility to infection was assessed in neonate or adult mice, infected through the i.p., intragastric (i.g.), or oral routes, by recovery of colony-forming units (CFUs) of *S. schenckii* from the lungs, liver, spleen, kidneys, and heart. In younger animals, susceptibility to infection was increased through the i.p. and i.g. routes when animals received the lower inoculum (6×10^6 *S. schenckii* conidia), with recovery of CFUs from 91 and 100 % of the animals, respectively, whereas the oral inoculation of the higher (2×10^7 conidia) and lower inoculums by gavage resulted in recovery of CFUs from only 21 and 73 % of the animals, respectively. On the other hand, adult mice were susceptible to infection through the i.p. and i.g. routes only. These results suggest that the gastrointestinal tract can also be one of the ways by which the fungus penetrates the host organism, although the lack of infection by the oral route in adult mice weights against the practical significance of such findings.

Highlighting the importance of T-cell-mediated immunity in this mycosis, congenitally athymic (nu/nu) mice showed greater susceptibility to disease and high mortality rates when infected with *S. schenckii* (Dickerson et al. 1983; Lei et al. 1993). Upon intravenous (i.v.) infection with *S. schenckii*, nu/nu mice were significantly more susceptible than their nu/+ littermates by both lethality and liver CFU counts. Additionally, as measured by liver CFU counts, thymus transplants from normal neonates conferred significant protection on nu/nu mice (nu/thy), and immunization with heat-killed *S. schenckii* yeasts enhanced the resistance of nu/thy and nu/+ mice. By contrast, immunization of nu/nu mice sharply increased their susceptibility. Last, the delayed-type hypersensitivity (DTH) response to a *S. schenckii* antigen showed that the resistance of immunized nu/thy mice mirrored the recovery of T lymphocyte function (Dickerson et al. 1983).

To investigate the *S. schenckii*-induced cell-mediated immune response, Carlos (1989) developed a model of systemic infection where lung, spleen, and liver commitment was accompanied by a granulomatous inflammatory response following the i.v. inoculation of 10^7 *S. schenckii* yeast cells into the retro-orbital plexus of Swiss mice. The general condition of mice worsened as the disease progressed until the 4th week after infection, as shown by marked fungal multiplication in the organs, progressive weight loss, diffuse granulomatous lesions with focal points of suppuration, and, ultimately, death. A state of depressed cell-mediated immune response, with reduced lymphocyte proliferation and DTH plus diminished interleukin (IL)-1 and tumor necrosis factor (TNF)-α release by peritoneal macrophages, was found between the 4th and 5th weeks after infection and could account, at least partially, for animal susceptibility. Beginning at the 5th week after infection,

surviving animals started to recover and gain back weight, which was accompanied by decreasing fungal loads and lesions stabilizing as chronic compact granulomas without suppuration foci. Of note, the infection's resolution was mirrored by a significant increase in cell-mediated immune response.

In another experimental model, the i.v. injection of 5×10^6 *S. schenckii* conidia into the retro-orbital plexus of mice allowed better recovery of CFU from a number of different organs, with results being more reproducible than those observed for either the i.p. or the i.v. injection via the tail vein (Fernandes et al. 1999). Studying the virulence of *S. lurei* in a murine model of disseminated infection, Fernández-Silva et al. (2012) showed that when OF-1 mice were intravenously challenged with two different inoculums (2×10^5 and 2×10^7 CFU/animal), only the highest resulted in animal death, which was observed between days 12 and 16 post-infection, with liver and spleen being the most affected organs. Arrillaga-Moncrieff et al. (2009), also using an OF-1 murine model, performed a comparative study on the pathogenicity of five *Sporothrix* species of clinical interest, namely *S. albicans*, *S. brasiliensis*, *S. globosa*, *S. mexicana*, and *S. schenckii s str*. The authors tested two strains of each species and two inoculums for each strain (2×10^7 and 2×10^4 conidia/animal) through the intravenous route and found that, when using the lower inoculum, mortality was caused by only one strain of *S. brasiliensis*, whereas both strains of *S. brasiliensis* and *S. schenckii* induced mortality when using the higher inoculum. Their results showed *S. brasiliensis* to be clearly the most virulent species in terms of mortality, tissue burden, and tissue damage, followed by *S. schenckii* and then *S. globosa*; in this particular study, *S. mexicana* and *S. albicans* showed low or no virulence.

7.3 Innate Immune Response

Despite its restricted specificity compared with the adaptive immune system, the innate immune system is capable of effectively distinguishing host cells (self) from pathogens (non-self) and serves as a first line of defense capable of rapidly reacting to insults. In recent years, the expanded knowledge regarding receptor complexity and the pivotal role played by innate immune cells in providing the signals that trigger and direct the adaptive immune response has brought special attention to the field (Abdelsadik and Trad 2011; Hamad 2012).

7.3.1 Reactive Oxygen and Nitrogen Species in the Anti-S. schenckii Host Response

Macrophages are central to an effective immune response against pathogens and, for many fungal diseases, they are the major cell population implicated in host

protection, primarily by their ability to eliminate the invading fungal pathogen through phagocytosis. Upon phagocytosis, macrophages activate signaling pathways leading to antigen processing, intracellular trafficking, and presentation to adaptive immune cells, triggering an inflammatory response (Underhill and Ozinsky 2002; Pluddemann et al. 2011). When activated, these cells show an accelerated metabolic rate, increased motility, and phagocytic activity; they also can secrete enzymes, components of the complement, coagulation factors, and various cytokines (Palladino et al. 2003). Macrophages also act as a first line of defense for the host by releasing a great number of factors, including reactive oxygen (ROS) and nitrogen species (RNS), such as hydrogen peroxide (H_2O_2) and nitric oxide (NO), respectively, known as a powerful mediators of inflammation and other types of immune responses (Laskin et al. 1994; Parslow and Bainton 1997; Guzman-Beltran et al. 2012). In turn, NO, together with oxygen radicals, contributes to the cytotoxic activity of phagocytes, including macrophages, toward certain bacteria, protozoan parasites, fungi, and viruses.

ROS and RNS may play different roles depending on their relative concentrations and the cell sites accessible to these species. The importance of ROS in macrophage microbicidal activity was suggested by the evidence that peritoneal macrophages produce ROS upon activation or exposure to pathogens (Sasada and Jonhston 1980) and that macrophage activity is inhibited by free radical scavengers, such as superoxide dismutase, an enzymatic superoxide ($O_2\bullet^-$) scavenger, and catalase, an enzymatic H_2O_2 scavenger (Takao et al. 1996; Lee and Yea 2000). NO production by macrophages may alter the balance between host defense mechanisms and pathogen virulence, thereby decreasing the host's susceptibility to infection. ROS are used in the body as oxidative cytotoxic agents produced by phagocytic cells during the respiratory burst induced by infection. The majority of ROS, including H_2O_2 and singlet oxygen ($^1O_2$), are produced by two pathways: via the phagocyte nicotinamide adenine dinucleotide phosphate (NADPH) oxidase or the hypoxanthine metabolism. Both H_2O_2 and $O_2\bullet^-$ can function independently as cytotoxic agents or form other toxic molecules like the hydroxyl radical (•OH) or hypochlorous acid (HOCl), or peroxynitrite ($ONOO^-$) in the presence of NO (Ramasarma 1990).

Yeast cells from naturally virulent dimorphic fungi, in contrast to opportunistic fungi, survive phagocytosis by neutrophil granulocytes in vitro: although naturally virulent yeast forms ingested by polymorphonuclear cells (PMNs) generally trigger a respiratory burst comparable to that induced by opportunists, they are less susceptible to H_2O_2 and other leukocyte microbicidal products (Schaffner et al. 1986). Ergosterol peroxide production by *S. schenckii* may play a role in protecting this fungus from the oxidative burst of PMNs. Sgarbi et al. (1997), using ^1H and ^{13}C nuclear magnetic resonance and high-resolution mass spectrometry, identified ergosterol peroxide from the yeast form of *S. schenckii* that reverted back to ergosterol upon contact with an *S. schenckii* enzyme extract. We thus suggested the *S. schenckii*-produced ergosterol peroxide, a presumed product of the H_2O_2-dependent enzymatic oxidation of ergosterol, to be formed as a protective agent to evade ROS produced during phagocytosis (Fig. 7.2). The production of ergosterol

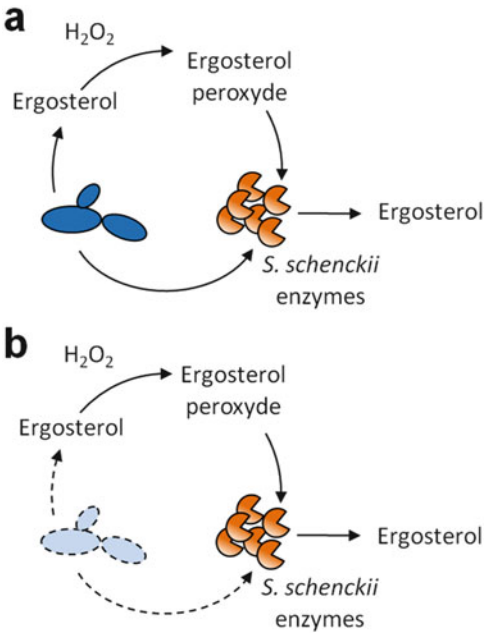

Fig. 7.2 The H_2O_2-dependent enzymatic oxidation of ergosterol yields ergosterol peroxide, which was showed to revert back to ergosterol upon contact with a *Sporothrix schenckii* enzyme extract, thereby suggesting that ergosterol peroxide synthesis by *S. schenckii* yeasts may be used as an escape mechanism from the oxidative burst of PMNs during phagocytosis. We hypothesize that ergosterol peroxide and its reversion-associated enzymes could be actively secreted by live *S. schenckii* yeasts inside the phagosome (**a**) or both products could be released from dying fungal forms upon pathogen killing during phagocytosis (**b**), or even a combination of the two mechanisms. *PMNs* polymorphonuclear cells (reproduced with modifications from Carlos et al. 2003, with permission of John Wiley and Sons)

peroxide by a pathogenic fungus, reported for the first time in our work, suggests that a possible detoxification reaction may also represent a virulence factor. Since $^1O_2$, another product resulting from the oxidative burst of PMNs (Clark 1999), may not be involved in the peroxidation of ergosterol (Bates et al. 1976), it seems that survival of virulent yeast forms upon contact with PMNs depends on additional escape mechanisms besides the one leading to the synthesis of ergosterol peroxide.

Differences in cell wall composition have been shown to play a role in the virulence of medically important fungi such as *Candida albicans* (Mencacci et al. 1994) and *Cryptococcus neoformans* (Kozel 1995). Although the cell wall composition of *S. schenckii* is well known, its role in the immune response against sporotrichosis is poorly understood. The effect of two cell wall extracts (an alkali-insoluble fraction and a lipid extract) and the exoantigen obtained from the yeast form of *S. schenckii* in the macrophage–fungus interaction was assessed in regard to the generation of H_2O_2 (Pick and Keisari 1980, with modifications) and NO (Green et al. 1982) by peritoneal macrophages. We found that H_2O_2 release was mainly

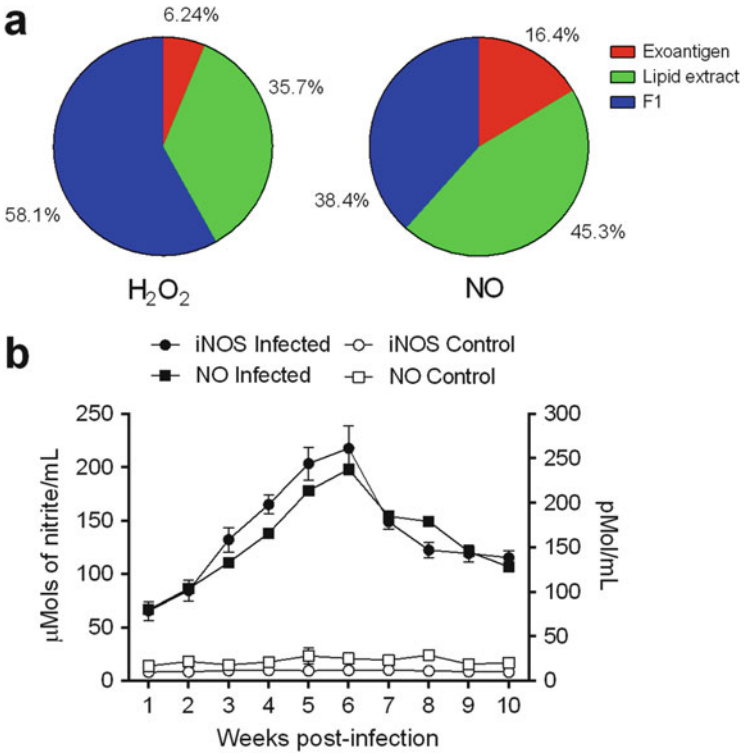

Fig. 7.3 Reactive oxygen and nitrogen species induced in response to *Sporothrix schenckii*. Peritoneal macrophages from *S. schenckii* (1099-18 clinical isolate)-infected Swiss mice were cultured for 24 h with the indicated derivatives from the *S. schenckii* yeast cell wall and the ex vivo release of H_2O_2 and NO was assessed in the culture supernatant by the phenol red and Griess method, respectively (**a**). Peritoneal macrophages from Swiss mice infected with the *S. schenckii* 1099-18 clinical isolate were cultured for 24 h with the *S. schenckii* exoantigen, and the iNOS activity (*right y axis*) and NO production (*left y axis*) were assessed in the culture supernatant by ionic exchange chromatographic detection of the radiolabeled L-arginine to L-citruline conversion and the Griess assay, respectively (**b**). Results are reported as the percentage of the means' sum from five independent experiments of each reactive species induced by each *S. schenckii* derivative (**a**) or the mean ± standard deviation of four independent experiments (**b**). *F1* Alkali-insoluble fraction; *iNOS* inducible nitric oxide synthase, *NO* nitric oxide (panel **b** was reproduced with modifications from Maia et al. 2006, with permission of Springer)

induced by the alkali-insoluble fraction (F1, Kanetsuma and Carbonell 1970), whereas NO release was higher in response to the lipid extract (Svennerholm and Fredman 1980); the exoantigen was a comparatively poor inducer of both H_2O_2 and NO (Fig. 7.3a) (Carlos et al. 2003). Other experiments from our laboratory have shown that the F1 fraction is able to induce a granulomatous reaction, whereas the exoantigen is known to elicit a humoral response (unpublished data). Granuloma formation, thought to result from a T-cell-dependent inflammatory response, is an essential component of the normal host defense and a critical event in the immune response against *S. schenckii* (Kauffmann 1999; Miranda et al. 2013).

We later investigated the role of the *S. schenckii* exoantigen in driving NO production through assessment of the NO synthase's (NOS) inducible isoform (iNOS), a NADPH-dependent enzyme that generates NO by oxidation of the terminal guanidine's nitrogen atoms from L-arginine (Kolb et al. 1994). The expression of iNOS is mainly regulated by cytokines, generally through activation of the iNOS gene promoter in murine macrophages (Bogdan 2001). NO and iNOS activity were assayed in cultures of peritoneal macrophages challenged with the *S. schenckii* exoantigen. Similar to previous studies, the highest NO levels were found between the 4th and 7th weeks post-infection, mirroring the increase in iNOS activity (Fig. 7.3b) (Maia et al. 2006), thus suggesting the production of NO to be a product of the iNOS activation by the fungus' exoantigen. How other components of *S. schenckii* contribute to iNOS induction remains to be further explored, but this same mechanism is likely to play a role.

7.3.2 Pattern Recognition Receptors Involved in S. schenckii Recognition

Compared with our current understanding of the adaptive immune system, we still know very little about the receptors and molecular mechanisms employed by the innate immune system for pathogen recognition and self–non-self discrimination. One of the best known classes of innate receptors are the pattern recognition receptors (PRRs), which are able to detect and respond to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (Kanneganti et al. 2007; Vera-Cabrera et al. 2012). At least four major families of PRRs operate cooperatively to recognize pathogens and stress signals produced by cells during infection or cell damage, namely Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), C-type lectin-like receptors (CLRs), and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). Among these, the CLRs (e.g., Dectin-1, Dectin-2, Mincle, dendritic cell [DC]-specific intercellular adhesion molecule 3-grabbing non-integrin [DC-SIGN], and the mannose receptor [MR]), the scavenger receptors (e.g., CD5 and CD36), Galectin-3, and TLRs are the main PRR families implicated in fungal PAMP recognition (Romani 2011).

7.3.2.1 Toll-Like Receptors

TLRs were first identified based on their ability to control fungal infections in *Drosophila melanogaster* and hence are named after the *Drosophila* Toll protein. In mammals, TLRs comprise 13 members, of which TLR1-9 are conserved between humans and mice, while TLR10 is non-functional in mice and TLR11–13 are not present in the human genome (Brown 2011; Sasai and Yamamoto 2013). Engagement of TLRs by their agonists promotes dimerization of the extra and intracellular

receptor domains, driving signal transduction primarily through recruitment of the myeloid differentiation primary response protein 88 (MyD88), but also of other adaptor proteins; this ultimately leads to activation of the adaptive immune system, whether directly owing to TLR expression on T and B cells, or indirectly by induction of major histocompatibility complex class II (MHCII) and co-stimulatory molecule expression on DCs (Abdelsadik and Trad 2011; Song and Lee 2012). Surface immune recognition of most, but not all, fungi is carried over by TLR4 (mannans of several fungal species, *C. albicans* O-linked mannans and *C. neoformans* glucuronoxylomannan [GXM]) and the TLR2/1 and TLR2/6 heterodimers (β-glucans and mannans of several fungal species, *C. albicans* phospholipomannans and *C. neoformans* GXM). Furthermore, the microbe's phagosome degradation may expose additional PAMPs within the phagosome, such as nucleic acids, allowing further pathogen recognition through the TLRs 3, 7, and 9. It is generally accepted that, during fungal infections, TLRs preferentially mediate the development of type 1 T helper (Th1) responses, although they may also play a direct role in the induction of Th17 responses, and TLR2 may promote T-regulatory (Treg) cell differentiation, thereby limiting Th17 or Th1 cell differentiation (Bourgeois and Kuchler 2012). Nevertheless, as for other microbial pathogens, fungi usually carry multiple classes of PAMPs, and their recognition therefore likely involves several PRRs from the same or different families; also, although many TLRs are able to recognize fungi, they are not the primary receptors driving pathogen engulfment, as discussed later.

TLR4 has already been demonstrated as playing a role in sporotrichosis. Sassá et al. (2009) showed that, after challenge with a *S. schenckii* lipid extract, the ex vivo release of NO, TNFα and IL-10 by peritoneal exudate cells (PECs) from *S. schenckii*-infected TLR4-deficient C3H/HeJ mice was markedly impaired when compared with the TLR4-normal C3H/HePas mice, suggesting the involvement of TLR4 in the recognition and subsequent activation of PECs in response to the *S. schenckii* lipid components. Consistent with the results found for cytokine production, translocation into the nucleus of the transcription factor nuclear factor-κB (NFκB) was also impaired in TLR4-deficient mice. A revisit of the previous model showed that macrophages are largely dependent on TLR4 for inflammatory activation, as measured by H_2O_2, IL-1β, and IL-6 ex vivo release in response to the *S. schenckii* lipid extract, and that in the absence of TLR4 signaling, increased transforming growth factor (TGF)-β release may be one of the contributing factors for the abrogated inflammatory activation of PECs during mice sporotrichosis (Sassá et al. 2012).

Interestingly, in the study of Sassá et al. (2012) above, the lipid extract-induced IL-1β and IL-6 release, while sensibly diminished on infected TLR4-deficient mice, was still significantly increased in these animals, suggesting the activation of other, TLR4-independent, signaling pathways. By contrast, the lipid-induced H_2O_2 release by PECs from infected TLR4-deficient mice was completely abrogated; coupled with the fact that the exoantigen was not able to induce either H_2O_2 or IL-6 release and only a minor increase in IL-1β by PECs from infected TLR4-normal mice, this suggests that H_2O_2 induction in this system is solely triggered by the

fungus' lipid compounds, rather than its exoantigen, exclusively in a TLR4-dependent way. Finally, TGF-β release was found to be increased only in culture supernatants of PECs from infected TLR4-deficient mice in response to both fungal products, but mainly the lipid. It seems that TLR4 engagement by *S. schenckii* skews the immune system toward an inflammatory response by suppressing anti-inflammatory pathways that would otherwise be triggered. As a whole, these studies suggest a role for TLR4 in sporotrichosis as one of the innate immune receptors recognizing *S. schenckii*, primarily through its lipid compounds, and thereby inducing a pro-inflammatory response. Also, the inability of the exoantigen to elicit a potent inflammatory response is further evidence of its use by *S. schenckii* as an escape mechanism conveniently released into the external environment as it grows (Fig. 7.4).

Phagocytosis, mainly carried out by professional phagocytes such as macrophages, DCs, mast cells, monocytes, and neutrophils, constitutes a first line of

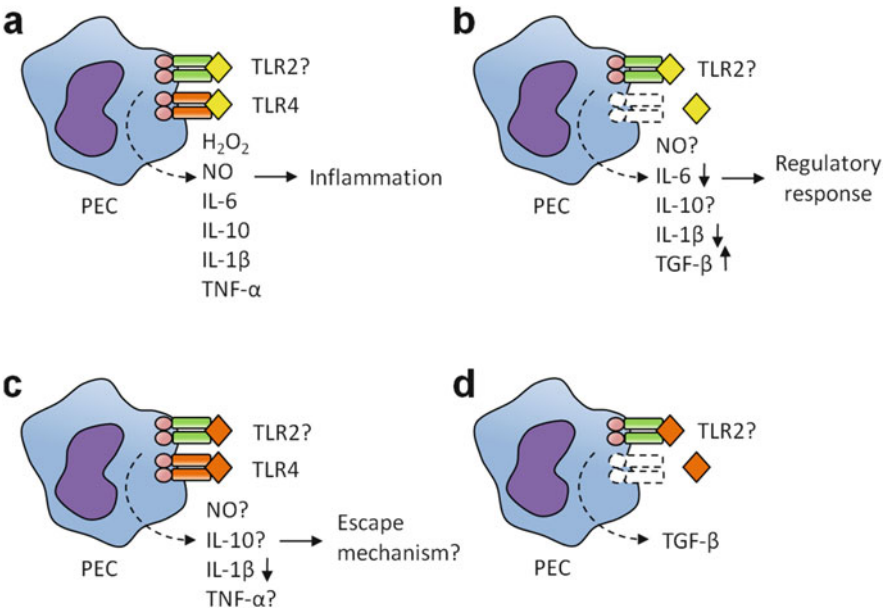

Fig. 7.4 During *Sporothrix schenckii* infection, TLR4 engagement by the lipid components of the fungus seems to skew the immune system toward an inflammatory response by suppressing TGF-β-dependent (maybe TLR2-mediated) anti-inflammatory pathways that would otherwise be triggered (**a** and **b**). On the other hand, the fungus' exoantigen may be used as an escape mechanism, given its inability to induce the release of the pro-inflammatory mediators H_2O_2 and IL-6 and only poor induction of IL-1β (**c** and **d**). In addition, although largely dependent on TLR4, the lipid extract-induced IL-1β and IL-6 release by PECs is not entirely TLR4-dependent (**b**), in contrast to the H_2O_2 release, which seems solely triggered by the fungus' lipid compounds, rather than its exoantigen, exclusively in a TLR4-dependent way (all panels). *IL* interleukin, *PEC* peritoneal exudate cell; *TGF* transforming growth factor, *TLR4* Toll-like receptor 4; *TLR2* Toll-like receptor 2

defense for the host, allowing antigen sampling and processing from invading pathogens. Although fungal phagocytosis is most efficient when the fungi are opsonized (e.g., by antibodies, complement, or soluble secreted PRRs), various membrane-bound PRRs, including the scavenger receptor class F member 1 (SCARF1), MR, DC-SIGN, and Dectin-1, are able to mediate the uptake of non-opsonized fungi (Brown 2011). Phagocytosis is commonly mediated simultaneously by multiple receptors, including Fc receptors, complement receptors, scavenger receptors, and various integrins and PRRs, some of which are tasked directly with phagocytosis while others primarily participate in binding or to increase the internalization efficiency (Underhill and Ozinsky 2002). Engagement of the Fcγ receptor (FcγR, i.e., a receptor for the Fc portion of immunoglobulin [Ig]-G antibodies) triggers both phagocytosis and inflammatory signal transduction. Among the various PRRs, the MR is exclusively a phagocytic receptor, lacking inflammatory signaling capabilities, although it can modulate signaling by costimulatory-only PRRs, such as TLRs, which are important in potentiating phagocytosis through other receptors. Dectin-1 on the other hand can initiate both phagocytosis and inflammatory signaling. Interestingly, TLRs 3, 7, 8, and 9 are specifically localized in phagosomes and endosomes and thus require previous phagocytosis in order to be engaged by their ligands, whereas TLR2 and TLR4 are normally found on the plasma membrane and can bind their ligands prior to phagocytosis initiation (Moretti and Blander 2014).

We recently reported a role for TLR2 in the phagocytosis of *S. schenckii* and in the production of inflammatory mediators by macrophages (Negrini et al. 2013). In order to assess the role of some of the components of *S. schenckii* in mediating the fungus' attachment and internalization, macrophages were exposed to two *S. schenckii* yeast extracts, a soluble antigen extract (SolAg) and a cell wall lipid extract (LipAg), prior to contact with the fungus. The percentage of macrophages containing internalized yeasts was extremely low in TLR2$^{-/-}$ mice (5.6 % ± 0.2) when compared with the wild-type (WT) mice (90.1 % ± 1.8). When macrophages were pre-incubated with LipAg or SolAg, internalization of *S. schenckii* was drastically diminished in WT and further reduced in TLR2$^{-/-}$ mice, although still not completely (Fig. 7.5). Contrary to uptake of *Candida* and the TLR2 classical ligand zymosan by macrophages (Underhill and Ozinsky 2002; Brown 2011), it seems that, in the absence of antibody or complement opsonization, phagocytosis of *S. schenckii* through other receptors is highly dependent on co-stimulation by TLR2. Additionally, both fungal components (i.e., the soluble and lipid extracts) were shown to play a significant part in this process, providing an explanation for previous observations showing that the *S. schenckii* lipid extract is able to inhibit the fungus uptake by peritoneal macrophages (Carlos et al. 2003). Furthermore, the reduction in *S. schenckii* internalization by WT and TLR2$^{-/-}$ macrophages pre-incubated with SolAg and LipAg, plus the fact that it is not complete in either, indicates that (1) both extracts have components recognized by other receptors, phagocytic or not, besides TLR2, and (2) additional receptors not engaged by either extract participate in the phagocytosis process. A multitude of membrane-bound receptors have been implicated in the recognition of unopsonized fungi and fungal

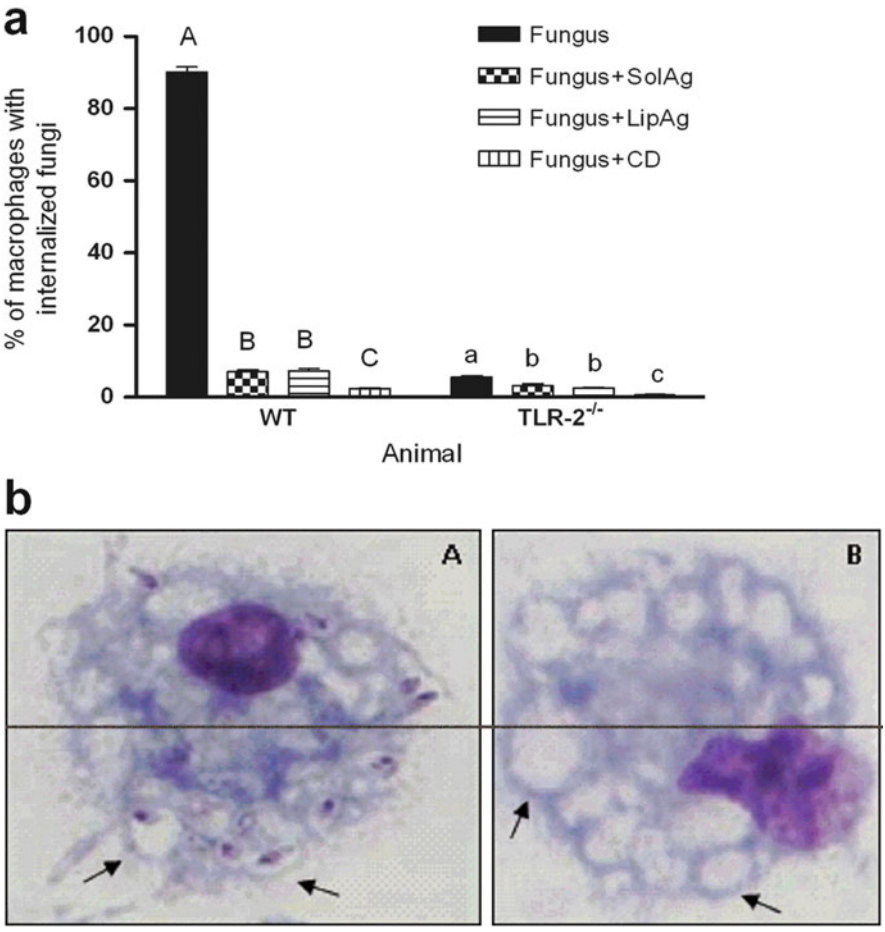

Fig. 7.5 In the absence of antibody or complement opsonization, phagocytosis of *Sporothrix schenckii* is highly dependent on co-stimulation by TLR2. Peritoneal macrophages were incubated for 2 h with an *S. schenckii* (1099-18 clinical isolate) yeast suspension (fungus) with or without prior incubation with the fungus' SolAg, LipAg, or the phagocytosis inhibitor CD, as indicated (**a**). *Arrows* indicate internalized yeasts by macrophages from WT mice (**b**, *box A*) or a large number of vacuoles and absence of yeast internalization by macrophages from TLR2$^{-/-}$ mice (**b**, *box B*). The efficiency of fungal internalization was determined in Giemsa-stained samples by counting 200 macrophages per sample by optical microscopy under 100 × magnification. Statistical significance was determined by two-way ANOVA using a 95 % confidence interval. Distinct capital or lower case letters represent statistically significant differences between the tested conditions within the WT or TLR2$^{-/-}$ groups, respectively. Results are presented as the mean ± standard deviation of the percentage of macrophages containing internalized fungi from three independent experiments. *ANOVA* analysis of variance, *SolAg* soluble antigenic extract; *LipAg* lipid extract; *CD* cytochalasin D; *WT* wild type; *TLR2*: Toll-like receptor 2 (reproduced with modifications from Negrini et al. 2013, with permission of Informa Healthcare)

components (Brown 2011) also found on *S. schenckii* (see Chap. 3) and could therefore be implicated in the residual *S. schenckii* recognition by macrophages from TLR2$^{-/-}$ mice; these include mainly the MR (mannan), Dectin-1 (-β-1,3-glucan), CD14 (mannan), and TLR4 (mannan), the latter already suggested to play a role in *S. schenckii* recognition (Sassá et al. 2009, 2012).

7.3.2.2 NOD-Like Receptors and the Inflammasome

NLRs are cytosolic PRRs forming penta or heptameric multiprotein complexes called inflammasomes. Owing to NLRs, microorganisms that evade detection by surface receptors can find a second line of defense within the host cells' cytosol. Multiple distinct inflammasomes each contain a key member of the NLR superfamily charged with conferring specificity for recognition of a particular microbial product (Philpott et al. 2000; Yang et al. 2012). In addition to an NLR family member, the inflammasome comprises the adaptor protein apoptosis-associated speck-like protein containing a carboxy-terminal CARD (ASC) and the enzyme caspase-1. The recognition of DAMPs or PAMPs by the NLR promotes recruitment of ASC, which in turn activates caspase-1, forming the inflammasome (Kumar et al. 2011; Paiva-Oliveira et al. 2012), the activation of which induces the production of pro-inflammatory cytokines and recruitment of immune cells to the tissue (Davis et al. 2011; Strowig et al. 2012). The inflammasome acts as an important driver of inflammation by promoting the maturation of pro-IL-1β and pro-IL-18 into their active forms, which in turn contribute to host defense against microbial infections by increasing the antimicrobial properties of phagocytes and triggering Th1 and Th17 responses (Veerdonk et al. 2011). Furthermore, the inflammasome can also defend the host from invading agents by promoting cytoplasmic pyroptosis, a caspase-1-dependent programmed cell death leading to pore formation, cytoplasm swelling and, thereby, osmotic cell lysis and leakage of intracellular contents (Malireddi and Kanneganti 2013; Wellington et al. 2014).

The inflammasome has been shown to participate in responses to a broad spectrum of infectious agents, including bacterial pathogens such as *Chlamydia pneumonia, Francisella novicida* (Shimada et al. 2011; Pierini et al. 2014), fungi like *C. albicans, Aspergillus fumigatus, Paracoccidioides brasiliensis*, and *Microsporum canis* (Gross et al. 2009; Said-Sadier et al. 2010; Tavares et al. 2013; Mao et al. 2014), viruses (Ito et al. 2012; Rajan et al. 2011), and parasites (Dai et al. 2011; Lima-Junior et al. 2013; Gorfu et al. 2014), as well as damaged host cell products (Lamkanfi and Dixit 2014). Several studies have identified a fundamental role for the inflammasome in host defense during fungal infections. Mice deficient in inflammasome components have shown higher fungal colonization, increased fungal multiplication, lower survival, and diminished production of IL-1β and IL-18, as well as abrogated Th1 and Th17 responses, resulting in disease worsening (Hise et al. 2009; Veerdonk et al. 2011).

A role for inflammasome activation in response to *S. schenckii* has been recently suggested in a mouse model of *S. schenckii* systemic infection, where reduced

caspase-1 activity was found between the 4th and 6th weeks post-infection, accompanied by diminished IL-1β, IL-18, and IL-17 ex vivo release from a peritoneal macrophage-splenocyte co-culture challenged with the *S. schenckii* lipid extract or alkali-insoluble fraction (Gonçalves et al. 2015). Interestingly, such transitory reduction in caspase-1 activity occurred in a time-frame closely matching the period of increased fungal burden and immunosuppression previously reported in similar models (Carlos et al. 2009). By using C57BL/6 mice lacking key components of the inflammasome complex (i.e., the adaptor protein ASC and caspase-1), we are currently trying to clarify how inflammasome activation influences the immune response against *S. schenckii*. Preliminary results show that inflammasome-deficient ($ASC^{-/-}$ and caspase-$1^{-/-}$) mice were generally more susceptible to the *S. schenckii* infection, as shown by higher spleen fungal loads and lower splenic index and spleen weight than the WT mice. Both knockout mice lineages were also less prone to release the pro-inflammatory mediators NO, IL-1β, IL-18, and IL-17 following ex vivo challenge with the *S. schenckii* lipid extract (unpublished data). Surprisingly, given the well-known ability of IL-18 to induce interferon (IFN)-γ production by innate and adaptive cells (Guo et al. 2012), we found that IFN-γ levels were similar between WT and knockout mice. Taken together, these studies suggest that an intact inflammasome is essential for optimal *S. schenckii* control, supposedly by stimulating a protective pro-inflammatory response (Fig. 7.6).

Macrophage Activation Profiles: After initial fungi recognition, macrophages can adopt a variety of phenotypes, resulting from their broad tissue distribution and adaptation to diverse microenvironments, besides numerous endogenous and exogenous stimuli (Gordon 2003). Macrophages are pivotal for proper immune responses against a variety of pathogens, playing different roles in response to different microorganisms and activating pathways. They can be divided into "classic" or "alternatively" activated populations, also known as M1 and M2 macrophages, respectively. M1 cells can lead to tissue injury and contribute to the pathogenesis of inflammatory diseases through production of high levels of IL-12 and marked iNOS upregulation (Mantovani et al. 2004; Hao et al. 2012), whereas M2 cells produce high levels of IL-10, upregulate arginase-I, and promote angiogenesis, tissue remodeling, and repair (Munder et al. 1998; Martinez et al. 2009). Our laboratory has previously reported a differential role for M1 and M2 macrophages during the course of the *S. schenckii* experimental infection. We showed an increase in the frequency of macrophages (F4/80 + CD11b + cells) inside PECs during the 4th and 6th weeks post-infection compared with the 2nd and 8th weeks. Additionally, gating macrophages based on expression of CD197 and CD121b (M1 cells) or CD23 and CD206 (M2 cells) revealed a predominance of M2 cells throughout the experimental period. However, the *S. schenckii* cell wall peptide-polysaccharide (PPC)-challenged PECs had increased IL-12 and NO ex vivo release at the 2nd and 4th weeks post-infection, suggesting macrophages are activated toward the M1 phenotype early on during the course of the *S. schenckii* infection. On the other hand, the peak in arginase-I activity and in IL-10 and TGF-β ex vivo release at the later stages of the infection (6th and 8th weeks) suggested the

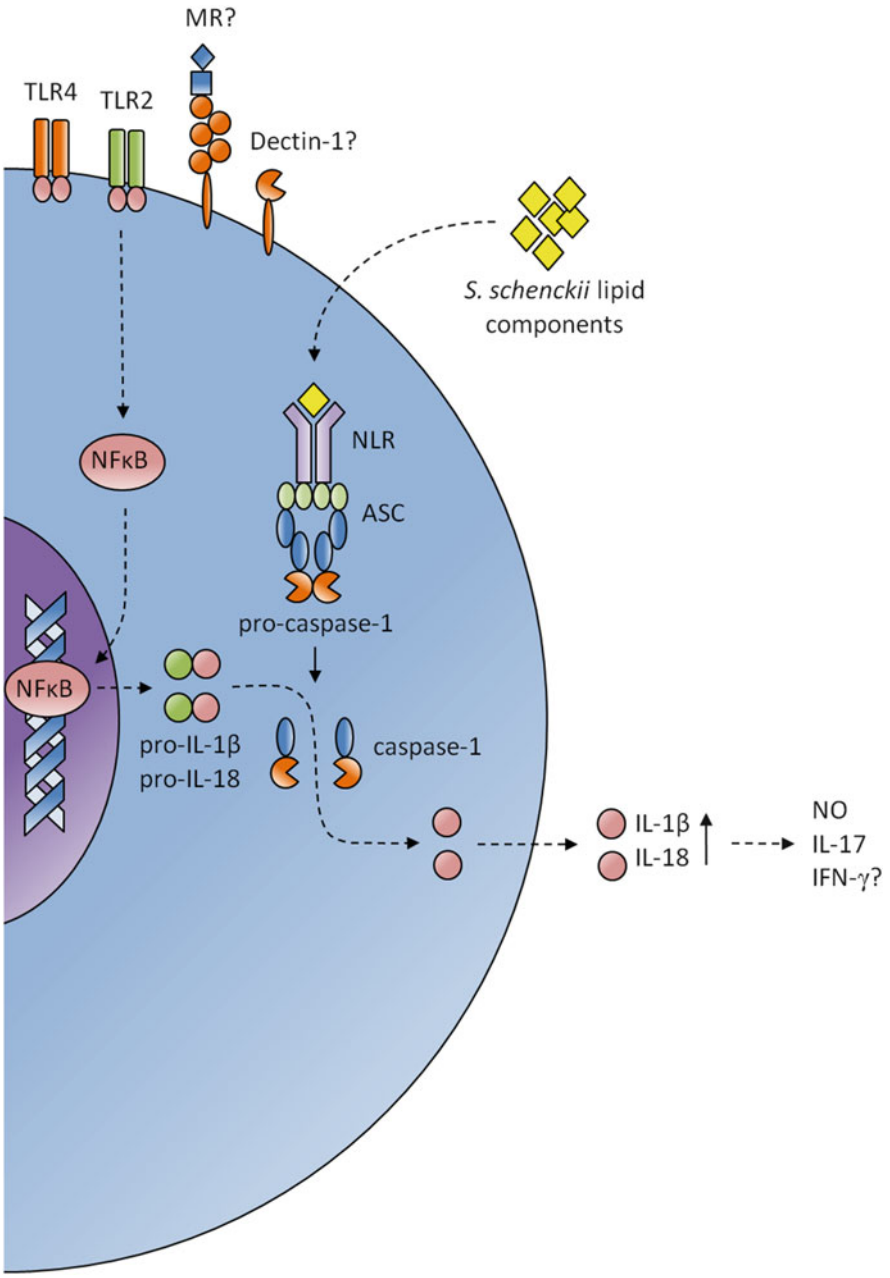

Fig. 7.6 Inflammasome activation by the *Sporothrix schenckii* lipid components may contribute to fungal control during sporotrichosis by stimulating a protective pro-inflammatory response driven by NO, IL-1β, IL-18, and IL-17. However, contrary to expected given the well known ability of IL-18 to induce IFN-γ production by innate and adaptive cells, IFN-γ production during mouse sporotrichosis seems to be induced in an inflammasome-independent way. Moreover, the inflammasome-mediated conversion of pro-IL-1β and pro-IL-18 into their active forms depends on a previous signaling able to trigger their expression, commonly through a surface PRR, we thus

predominance of the M2 macrophage population (Alegranci et al. 2013), consistent with their role in tissue remodeling and repair (Martinez et al. 2009).

Differential Dendritic Cell Activation may Determine the Following Adaptive Response: DCs also play a key role as an innate regulator of the balance between the pro- and anti-inflammatory responses required to augment or attenuate cell-mediated immunity (Segal 2007). Upon pathogen encounter, the cytokine profile released by DCs has a pivotal role in the initiation and type of the following adaptive immune response (Zhu et al. 2010). The specific role played by DCs in sporotrichosis is poorly understood. Uenotsuchi et al. (2006) showed that human monocyte-derived DCs (MoDCs) promoted different cell-mediated responses when stimulated with *S. schenckii* yeasts and conidia obtained from patients with visceral or cutaneous disease, and suggested such differences to be related to the p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) signaling pathways. DCs stimulated with the fungus from cutaneous origin were more potent in inducing a Th1 response than DCs stimulated with the fungus from a visceral origin, which caused only minimal DC activation and Th1 induction. In a previous study, we assessed the phenotypic and functional changes in bone marrow-derived DCs (BMDCs) stimulated with the *S. schenckii* whole yeast or exoantigen and found that recognition of both stimuli by DCs leads to development of a Th1/Th17 mixed response in vitro (Verdan et al. 2012).

7.4 Relationship Between Innate and Adaptive Responses in Sporotrichosis

Interactions between innate and adaptive immune responses enable effective host responses against a variety of infections and pathogenic agents. Several antifungal immune mechanisms are involved in the defense against *S. schenckii*. Previous studies have suggested that cell-mediated and humoral immunity both play important roles in host protection against this fungus (Scott and Muchmore 1989; Carlos et al. 1992; Tachibana et al. 1999; Nascimento and Almeida 2005). The outcome and clinical manifestations of *S. schenckii* infection are highly dependent on the immune status of the host: while immunocompetent individuals usually develop localized cutaneous forms, immunocompromised patients, including those infected

Fig. 7.6 (continued) propose TLR2 and TLR4, the ability of which to recognize *S. schenckii* has been shown previously, as possible sources of this first signal. Additional surface PRRs known to recognize fungal components also found on *S. schenckii* include the MR and Dectin-1, among others. *ASC:* apoptosis-associated speck-like protein containing a carboxy-terminal CARD, *IFN:* interferon, *IL:* interleukin, *MR:* mannose receptor, *NFκB:* nuclear factor κB, *NLR:* NOD-like receptor, *NO:* nitric oxide, *PRR:* pattern recognition receptor, *TLR4:* Toll-like receptor 4, *TLR2:* Toll-like receptor 2

with HIV, are predominantly affected by disseminated and systemic forms (Carlos et al. 2009). Furthermore, 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* (Siraishi et al. 1992; Moreira et al. 2015).

In an early study, a murine model of disseminated sporotrichosis was developed in which we showed that *S. schenckii*-infected mice had a depressed cell-mediated immune response, as indicated by lower DTH response against a *S. schenckii* yeast's soluble antigen extract, as well as lower lymphocyte proliferation in response to both the antigen and the mitogen concanavalin A (ConA) in vitro, between the 4th and 6th weeks post-infection. This same period was marked by the disease's worsening, body weight reduction, and mortality rate increase (Fig. 7.7a) (Carlos et al. 1992). Such a severe picture is likely to be at least partially related to the depressed cell-mediated immune response discussed before.

During an inflammatory response, cells of the innate and adaptive immune systems, including mast cells, neutrophils, lymphocytes, DCs, and macrophages, release a variety of mediators capable of triggering and/or enhancing specific aspects of the inflammatory response (D'Alessandro et al. 2003; Sargi et al. 2012). Among these, cytokines are of special interest. Cytokines are low-molecular-weight regulatory proteins or glycoproteins secreted in response to a number of stimuli by the host's immune and non-immune cells. In fungal infections, two independent cytokine pathways have been shown to drive inflammation: the IL-12–IFN-γ axis and the TNF-α-mediated pathway. IL-12 increases natural killer (NK) and T-cell proliferation and cytotoxic activity and also promotes the development of a Th1 immune response by inducing IFN-γ production by T cells (Puddu et al. 1997). IL-12 and IFN-γ drive Th1 development (Munder et al. 1998), though many researchers have shown that other cytokines, including IL-1α and TNF-α, are required for Th1 cells to produce maximal levels of IFN-γ (Ohteki et al. 1999; Robinson et al. 1985). TNF-α is a multifunctional cytokine with a key role in the pathogenesis of many infectious, acute, and chronic inflammatory conditions (Eigler et al. 1997) owing to its ability to induce the expression of other pro-inflammatory cytokines such as IL-1 and several chemokines (Okuda et al. 1996). Using a murine model of systemic infection, we showed that production of IL-1 and TNF-α by peritoneal macrophages from *S. schenckii*-infected mice was severely reduced between the 4th and 6th weeks post-infection (Carlos et al. 1994), resulting from and partly accounting for some of the features of the infection-induced immunosuppression found at this stage.

IFN-γ is also a powerful macrophage-activating factor (MAF), the local production of which in pathological states can help to perpetuate chronic disease (Funcht et al. 2001; Chen et al. 2005); besides, IFN-γ enhances ROS generation by regulating the expression of multiple enzymes (Hamilton et al. 1985). 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 pathogen growth. NO production is further increased in response to cytokines such as TNF-α, IFN-γ, and IL-12. Production of RNS induced by IFN-γ, alone or in combination with other cytokines,

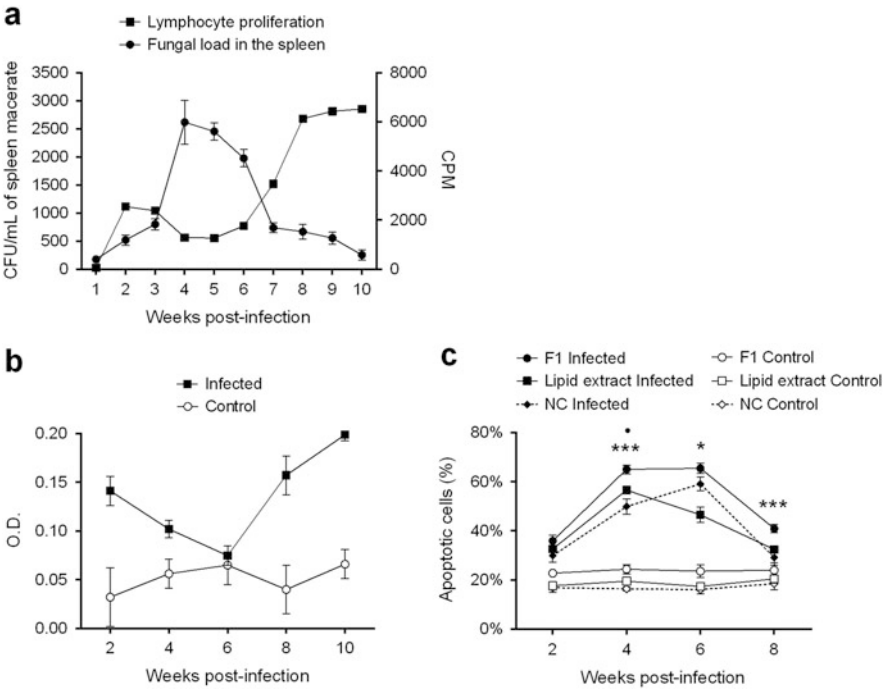

Fig. 7.7 General immune features in the course of experimental mouse sporotrichosis. Mice susceptibility to infection during sporotrichosis is inversely correlated to lymphocyte responsiveness following mitogen stimulation (**a**). At indicated times, the fungal load in the spleens of *Sporothrix schenckii* (1099-18 clinical isolate)-infected Swiss mice was assessed by CFU counting of an adequate dilution of the spleen macerate (**a**, *circles*); the lymphocyte proliferation was assessed by ^{3}H thymidine incorporation in cultures of lymph node cells from *S. schenckii*-infected mice stimulated for 48 h with 0.5 μg/well of ConA (**a**, *squares*). Results are presented as the mean ± standard deviation from five or three independent experiments. The production of anti-*S. schenckii* antibodies by *S. schenckii*-infected Swiss mice was determined by an in-house ELISA using peroxidase-conjugated goat anti-mouse IgG (**b**). Serum from infected (**b**, *squares*) and control (**b**, *open circles*) mice were assayed at 1/20 dilutions against the *S. schenckii* exoantigen at indicated times. Results are presented as the mean ± standard deviation of the O.D. at 490 nm from five independent experiments. The frequency of apoptotic cells was determined in peritoneal macrophages from *S. schenckii*-infected (**c**, *dark symbols*) or uninfected (**c**, *open symbols*) Balb/c mice cultured for 2 h in the presence of the *S. schenckii* lipid extract (**c**, *squares*), F1 (**c**, *circles*) or NC (**c**, *diamond shaped symbols*). Apoptosis was determined by flow cytometry using FLICA and PI staining. Statistical significance was determined by two-way ANOVA using a 95 % confidence interval. * ($p < 0.05$) and *** ($p < 0.001$) when comparing "F1 Infected" with "NC Infected," and • ($p < 0.05$) when comparing "Lipid extract Infected" with "NC Infected" on each time-point. Results are presented as the mean ± standard deviation of the percentage of cells undergoing apoptosis from three independent experiments. *ANOVA* analysis of variance, *CFU:* colony-forming units; *ConA:* concanavalin A; *CPM:* cynthilations per minute; *F1:* alkali-insoluble fraction, *FLICA:* fluorochrome inhibitor of caspases, IgG*:* immunoglobulin G, *NC:* negative control, *O.D:* optical density, *PI:* propidium iodide

may lead to suppression of T-cell responses in the microenvironment (Tachibana et al. 1999). IFN-γ also has an important regulatory role in the development of Th1 adaptive response against fungal pathogens through its ability to maintain IL-12 responsiveness in CD4+ T cells (Brüne et al. 1998); IL-12 in turn plays a critical role in the development of Th1 cells from naive T cells. Furthermore, IFN-γ enhances the antigen-presenting activity of macrophages, promoting additional stimuli for the expansion of the Th1 cell population. Therefore, IFN-γ production by antigen-presenting cells (APCs) not only leads to innate immunity enhancement, but it also establishes a link between the onset of innate immunity and the development of the following adaptive immune response.

IL-4 and IL-10 promote humoral immunity by stimulating the growth and activation of mast cells and eosinophils, the differentiation of B cells into plasma cells, and immunoglobulin class-switching to IgE. Moreover, these cytokines are able to inhibit macrophage activation, T-cell proliferation, and production of pro-inflammatory cytokines, thus making them major anti-inflammatory mediators (Bao and Cao 2014; Vazquez et al. 2015). At the early phase of a fungal infection, high IL-4 levels can delay the onset of the Th1 response by inhibiting IFN-γ production, whereas at advanced stages, IL-4 can limit infection development (Mencacci et al. 2000; Charalanpos and Roilides 2005). The ex vivo release of IL-4 was found to be increased only at the later stages of infection in a mouse model of systemic sporotrichosis, mirroring the increase in IgG antibody titers (Fig. 7.7b) (unpublished data) and in line with a preferential role for the Th2 response at more advanced stages of this infection found in a similar model (Maia et al. 2006). As is the case for the cross-talk between innate and adaptive responses, the interaction between cell- and antibody-mediated immunity is an important feature collaborating with host defense in experimental models of sporotrichosis.

7.5 Sporotrichosis, Apoptosis, and the Th17 Response

7.5.1 Apoptosis and the S. schenckii Infection

Apoptosis is a cell death mechanism in which the cell is commonly eliminated without triggering an inflammatory response, and it is characterized by rounding and reduction of the cell volume, chromatin condensation, and nuclear fragmentation, while cell integrity is maintained until the later stages of the process (Kroemer et al. 2009; Zeng et al. 2015). Infectious processes lead to activation of the immune system, with subsequent inflammation and cell death. Infection-induced apoptosis is associated with suppression of inflammation, sometimes as an escape mechanism employed by the invading pathogen, or with cell-mediated cytotoxicity for controlling the growth and spread of intracellular pathogens (Voll et al. 1997; Labbé and Saleh 2008).

Fernandes et al. (2008) showed that the *S. schenckii* infection-associated immunosuppression and susceptibility in a murine model of systemic infection were likely the result of increased NO production leading to spleen cell apoptosis and loss of responsiveness following stimulation by ConA, and high IL-10 and TNF-α production, events whose occurrence is a common trait of different types of infection (Navarre and Zychlinsky 2000). Such findings seem to provide an explanation for the characteristic depression of the cell-mediated immune response observed during the acute phase of sporotrichosis in previous studies (Carlos et al. 1992, 1994, 1999). However, NO can exhibit both pro- and anti-apoptotic properties depending on its concentration and source during an inflammatory response (Taylor et al. 2003). Nevertheless, evidence supporting the pro-apoptotic and immunosuppressive roles of NO in various cell types have predominated over the years (Blaylock et al. 1998; Laubach et al. 1995; Zumsteg et al. 2000) and it is now known that NO can induce T cells to lose responsiveness to mitogens (van der Veen 2001), an event that has been associated with apoptosis of these cells (Wu-Hsieh et al. 1998).

In accordance with the findings of Fernandes et al. (2008) above, a previous assessment of peritoneal macrophage apoptosis in a murine model of sporotrichosis showed that the peak in apoptotic cell death occurs during the 4th and 6th weeks post-infection, when the host is at its most compromised (Carlos et al. 1992, 1994). Furthermore, when peritoneal macrophages from infected animals were challenged with the *S. schenckii* lipid extract, the frequency of apoptotic cells was increased only on the 4th week post-infection when compared with unstimulated cells. However, when exposed to F1, the frequency of cells undergoing apoptosis was increased on the 4th, 6th, and 8th weeks post-infection (Fig. 7.7c). Considering that neither of the fungal components was able to induce apoptosis on cells from uninfected mice, it seems the increase in apoptotic cell frequency on cells from infected mice is due to the augmented stress state of these cells and/or Fas ligand (FasL) upregulation (Galluzzi et al. 2012), the latter already reported in response to a *C. neoformans* polysaccharide (Monari et al. 2005; Villena et al. 2008). If the above is true, then the maintained ability of the alkali-insoluble fraction to induce macrophage apoptosis until the later stages of the infection would be related to a more extended responsiveness to its components when compared with the lipid extract.

7.5.2 Sporotrichosis-Associated Th17 Response

Interestingly, infection-induced apoptosis, resulting in the simultaneous recognition of PAMPs and apoptotic cell products, has been implicated in the induction of Th17-cell development (Brereton and Blander 2010), whose role in sporotrichosis we are just starting to understand. Th17 cells are characterized by expression of the transcription factor retinoic-related orphan receptor γt (RORγt), one of the forms resulting from the alternative splice of the *rorc* gene, and are capable of producing,

besides IL-17 (IL-17A), also IL-17F, IL-22, and IL-21, among others (Huang et al. 2012). These cells play a key role in host defense against extracellular bacteria and fungi and in the maintenance of mucosal homeostasis, owing in great part to the pro-inflammatory actions of IL-17, which include neutrophil and Th1-cell recruitment and induction of pro-inflammatory cytokine production by epithelial cells (Cua and Tato 2010; Hernández-Santos and Gaffen 2012; van de Veerdonk et al. 2009a). The discovery of a *C. albicans* mannan, a class of polysaccharide of which the *S. schenckii* cell wall is largely made up (Shimonaka et al. 1975), capable of inducing IL-17 production in the absence of mitogenic stimuli through activation of the macrophage MR (van de Veerdonk et al. 2009b), suggested that Th17 cells could play a role in the immune response against sporotrichosis.

Recently, we demonstrated that recognition of the *S. schenckii* yeast or its exoantigen by DCs leads to development of a Th1/Th17 mixed response in vitro (Verdan et al. 2012). DCs were able to differentially recognize the *S. schenckii* yeast and its exoantigen, leading either to an IFN-γ- or to an IL-17-dominant profile when co-cultured with spleen lymphocytes, respectively. Since Th17 cells depend on TGF-β and IL-6 for differentiation and on IL-23 for maintenance and expansion (Korn et al. 2009), we decided to evaluate the ex vivo release of these cytokines by stimulated BMDCs. Interestingly, only the fungus' exoantigen, but not the whole yeast, was able to induce BMDCs to release significant amounts of both IL-6 and TGF-β, as well as IL-23. Challenge with the whole yeast led BMDCs to release increased amounts of IL-12 and IL-6, but not TGF-β. Therefore, it seems that the higher levels of TGF-β and IL-23 induced by the exoantigen, plus adequate levels of IL-6, in combination with a comparatively low induction of IL-12, which is well known to inhibit Th17-cell differentiation (Gocke et al. 2007), led to a more pronounced IL-17 release in our BMDC-spleen lymphocyte co-cultures (Fig. 7.8, left panel). This study suggested that the *S. schenckii* exoantigen is able to deviate the immune response from a Th1 to a Th17 pattern, in vitro at least, although the significance of this in vivo remains to be elucidated.

These results prompted us to investigate the Th1/Th17 response in vivo and its importance to the host defense against *S. schenckii* (Ferreira et al. 2015). We showed that the Th17 response in a mouse model of systemic infection manifests through the development of Th17 (RORγt single-positive) and Th1/Th17 (T-bet + RORγt+) mixed cells, as well as by an increased ex vivo release of both IL-17 and IL-22 by heat-killed yeast-challenged splenocytes (Fig. 7.8, right panel). However, contrary to expected given the scarcity of Th1 (T-bet single-positive) cells and only moderate numbers of Th1/Th17 cells, the amount of IFN-γ released was surprisingly large. We thus suggested CD3−CD49b+ NK cells, a population found to be greatly increased in infected mice, as a potential additional source of IFN-γ. Additionally, in order to assess the importance of the Th17 response to the host's ability to control the *S. schenckii* infection, mice were treated with an anti-IL-23p19 neutralizing monoclonal antibody (mAb), leading to a marked decrease in both IL-17 and IL-22 that was directly correlated with an impaired capacity to control the early *S. schenckii* infection. Nevertheless, anti-IL-23-treated animals were still able to survive the infection and ultimately eliminate the fungus. Taking

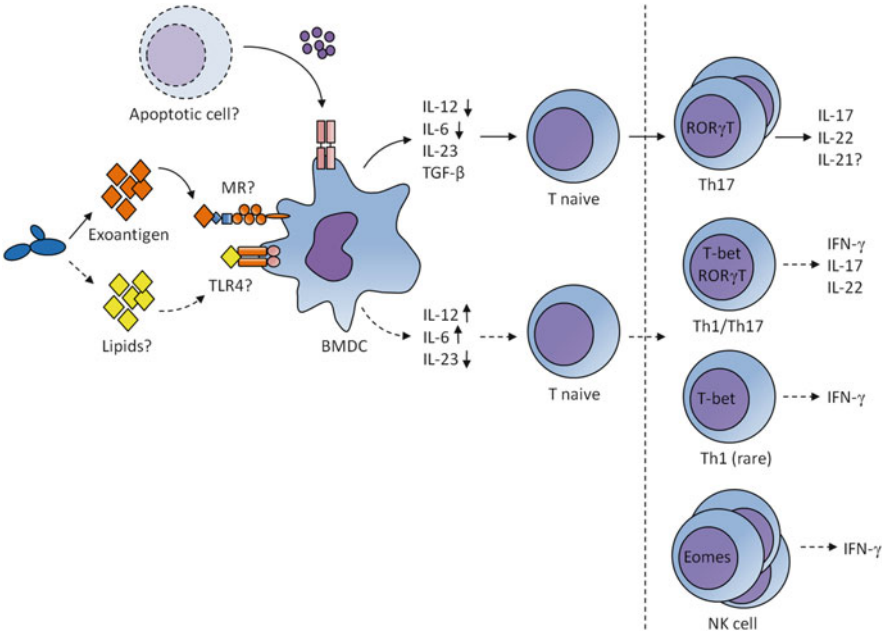

Fig. 7.8 *Sporothrix schenckii* exoantigen-challenged BMDCs release TGF-β and IL-23 plus moderate IL-6 levels, but only minimal quantities of IL-12, whereas *S. schenckii* whole yeast-challenged BMDCs release large amounts of both IL-12 and IL-6, but not TGF-β (*left panel*), leading to an IL-17 or IFN-γ dominant profile when co-cultured with spleen lymphocytes, respectively. We later found that the immune response against *S. schenckii* is marked, in vivo, by development of Th17 and NK cells, as well as Th1/Th17 mixed cells, but only rare Th1 cells (*right panel*). However, in contrast to the Th cell profile, the amount of IFN-γ released was very large. It is thus tempting to suggest a role for the *S. schenckii* exoantigen plus the infection-induced apoptosis (*left panel*) in driving the cytokine microenvironment toward a Th17-inducing profile (*solid lines*), while additional components present on the whole yeast contribute to a strong IFN-γ response driven by NK cells instead of Th1 cells (*dashed lines*). *BMDC* bone marrow-derived dendritic cell, *IL* interleukin, *IFN* interferon, *MR* mannose receptor, *NK* natural killer, *TGF* transforming growth factor, *Th17* type 17 T helper, *TLR4* Toll-like receptor 4

into account the results of Verdan et al. (2012) discussed above and these more recent findings, it is tempting to suggest a role for the *S. schenckii* exoantigen in driving the cytokine microenvironment toward a Th17-inducing profile, while additional components present on the whole yeast contribute to a strong IFN-γ response. Also worth noting is the possibility of NK cells, instead of Th1 cells, being major IFN-γ producers during sporotrichosis. Finally, given the recent emergence of innate lymphoid cells (ILCs) as potent cytokine producers capable of mirroring the profiles of the three major Th subsets, namely Th1, Th2, and Th17 (Artis and Spits 2015; McKenzie et al. 2014), future work focused on uncovering the immune mechanisms at play during sporotrichosis will greatly benefit from the assessment of such innate counterparts to Th cells.

7.6 Conclusions and Future Perspectives

Classically, murine models of sporotrichosis display a transitory state of depressed cell-mediated immunity during the disease's acute phase, which has been suggested to result from the NO-induced T-cell apoptosis and loss of responsiveness to mitogen stimulation. Given the more recent findings, it is tempting to speculate that the *S. schenckii* recognition through TLR4 leads mainly to an inflammatory response, while TLR2 is involved in regulating inflammation and could thereby be exploited as an escape mechanism by the fungus' exoantigen. Concomitantly, inflammasome activation and infection-induced apoptosis could both participate in driving a Th17-adaptive immune response against *S. schenckii*, whereas NK and other innate lymphoid cells would contribute through increased IFN-γ production. However, the exact contribution of each of the *S. schenckii* components, besides the role played by TLR2 and TLR4, as well as inflammasome activation, to the type of adaptive immune response in vivo remains poorly understood, with some conflicting findings along the way. Clearly, a better characterization of the antigen fractions employed in cell culture stimulation, or the use of highly purified or recombinant *S. schenckii* components, especially from the cell wall of the fungus, plus the use of genetically deficient animals for key components of the innate and adaptive machinery tasked with fungi recognition, will be of enormous help in solving these problems. We are currently assessing a number of major points regarding the innate recognition and the type of adaptive immunity produced in response to the *S. schenckii* infection, using somewhat more standardized animal models, which will certainly reduce the discrepancies arising from comparing results from different experimental settings.

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References

Abdelsadik A, Trad A (2011) Toll-like receptors on the fork roads between innate and adaptive immunity. Hum Immunol 72:1188–1193

Alegranci P, Ribeiro LCA, Ferreira, LS et al (2013) The predominance of alternatively activated macrophages following challenge with cell wall peptide-polysaccharide after prior infection with *Sporothrix schenckii*. Mycopathologia (1975 Print) 176:57–65

Arrillaga-Moncrieff I, Capilla J, Mayayo E et al (2009) Different virulence levels of the species of *Sporothrix* in a murine model. Clin Microbiol Infect 15(7):651–655

Artis D, Spits H (2015) The biology of innate lymphoid cells. Nature 517(7534):293–301

Bao Y, Cao X (2014) The immune potential and immunopathology of cytokine-producing B cell subsets: a comprehensive review. J Autoimmun 55:10–23

Bates ML, Reid WW, White JD (1976) Duality of pathways in the oxidation of ergosterol to its peroxide in vivo. J Chem Soc Chem Comm 44–45

Blaylock MG, Cuthbertson BH, Galley H et al (1998) The effect of nitric oxide and peroxynitrite on apoptosis in human polymorphonuclear leukocytes. Free Radic Biol Med 25(6):748–752

Bogdan C (2001) Nitric oxide and the immune response. Nat Immunol 2:907–916

Bourgeois C, Kuchler K (2012) Fungal pathogens-a sweet and sour treat for toll-like receptors. Front Cell Infect Microbiol 2:142

Brereton CF, Blander JM (2010) Responding to infection and apoptosis - a task for TH17 cells. Ann N Y Acad Sci 1209:56–67

Brown GD (2011) Innate antifungal immunity: the key role of phagocytes. Annu Rev Immunol 29: 1–21

Brüne B, Von knethen A, Sandau KB (1998) Nitric oxide and its role in apoptosis. Eur J Pharmacol 26:261–272

Carlos IZ (1989) Contribuição ao estudo da infecção sistêmica pelo fungo *Sporothrix schenckii* em camundongos. Avaliação do processo infeccioso e seu inter-relacionamento com a resposta imune celular. Ribeirão Preto School of Medicine of Ribeirão Preto – USP (Thesis – Doctor) 104pp

Carlos IZ, Sgarbi DBG, Angluster J et al (1992) Detection of cellular immunity with the soluble antigen of the fungus *Sporothrix schenckii* in the systemic form of the disease. Mycopathologia 117:139–144

Carlos IZ, Zini MMC, Sgarbi DBG et al (1994) Disturbances in the production of interleukin-1 and tumor necrosis factor in disseminated murine sporotrichosis. Mycopathologia 27:189–194

Carlos IZ, Sgarbi DBG, Placeres MCP (1999) Host organism defense by peptide-polysaccharide extracted from the fungus *Sporothrix schenckii*. Mycopathologia 144:9–14

Carlos IZ, Sgarbi DBG, Santos GC et al (2003) *Sporothrix schenckii* lipid inhibits macrophage phagocytosis: involvement of nitric oxide and tumor necrosis factor-α. Scand J Immunol 57: 214–220

Carlos IZ, Sassá MF, Placeres MCP et al (2009) Current research on the immune response to experimental sporotrichosis. Mycopathologia 168:1–10

Charalanpos A, Roilides E (2005) Cytokines and fungal infections. J Hematol 129:583–596

Charoenvit Y, Taylor RL (1979) Experimental sporotrichosis in Syrian hamsters. Infect Immun 23(2):366–372

Chen GH, McDonald RA, Wells JC et al (2005) The gamma interferon receptor is required for the protective pulmonary inflammatory response to *Cryptococcus neoformans*. Infect Immun 73: 1788–1796

Clark RA (1999) Activation of the neutrophil respiratory burst oxidase. Infect Dis 179(Suppl 2): S309–S317

Cua DJ, Tato CM (2010) Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol 10(7):479–489

D'Alessandro T, Prasain J, Benton MR et al (2003) Polyphenols, inflammatory response, and cancer prevention: chlorination of isoflavones by human neutrophils. J Nutr 133:3773S–3777S

Dai X, Sayama K, Tohyama M et al (2011) Mite allergen is a danger signal for the skin via activation of inflammasome in keratinocytes. J Allergy Clin Immunol 127(3):806–814

Davis BK, Wen H, Ting JP (2011) The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu Rev immunol 29:707–735

Dickerson CL, Taylor RL, Drutz DJ (1983) Susceptibility of congenitally athymic (Nude) mice to sporotrichosis. Infect Immun 40(1):417–420

Eigler A, Greten TF, Sinha B et al (1997) Endogenous adenosine curtails lipopolysaccharide-stimulated tumour necrosis factor synthesis. Scand J Immunol 45:132–139

Fernandes KSS, Mathews HL, Lopes-Bezerra ML (1999) Differences in virulence of *Sporothrix schenckii* conidia related to culture conditions and cell-wall components. J Med Microbiol 48:195–203

Fernandes KSS, Neto EH, Brito MMS et al (2008) Detrimental role of endogenous nitric oxide in host defense against *Sporothrix schenckii*. Immunology 123:469–479
Fernández-Silva F, Capilla J, Mayayo E et al (2012) Virulence of *Sporothrix luriei* in a murine model of disseminated infection. Mycopathologia 173(4):245–249
Ferreira LS, Gonçalves AC, Portuondo DL et al (2015) Optimal clearance of *Sporothrix schenckii* requires an intact Th17 response in a mouse model of systemic infection. Immunobiology. doi:10.1016/j.imbio.2015.02.009
Funcht DM, Fukao T, Bogdan C et al (2001) IFN-gamma production by antigen-presenting cells: mechanisms emerge. Trends Immunol 22:556–560
Galluzzi L, Vitale I, Abrams JM (2012) Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ 19(1):107–120
Gocke AR, Cravens PD, Ben LH et al (2007) T-bet regulates the fate of Th1 and Th17 lymphocytes in autoimmunity. J Immunol 78(3):1341–1348
Gonçalves AC, Maia DCG, Ferreira LS et al (2015) Involvement of major components from *Sporothrix schenckii* cell wall in the caspase-1 activation, nitric oxide and cytokines production during experimental sporotrichosis. Mycopathologia 179(1-2):21–30
Gordon S (2003) Alternative activation of macrophages. Nat Rev Immunol 3:23–35
Gorfu G, Cirelli KM, Melo MB et al (2014) Dual role for inflammasome sensors NLRP1 and NLRP3 in murine resistance to *Toxoplasma gondii*. mBio 5:1113–1117
Green LC, Wagner DA, Glogowski J et al (1982) Analysis of nitrate, nitrite and 15N in biological fluids. Anal Biochem 126:131–136
Gross O, Poeck H, Bscheider M (2009) Syk kinase signaling couples to the Nlrp3 inflammasome for anti-fungal host defence. Nature 459:433–436
Guo L, Junttila IS, Paul WE (2012) Cytokine-induced cytokine production by conventional and innate lymphoid cells. Trends Immunol 33:598–606
Guzman-Beltran S, Perez-Torres A, Coronel-Cruz C et al (2012) Phagocytic receptors on macrophages distinguish between different *Sporothrix schenckii* morphotypes. Microbes Infect 14: 1093–1101
Hamad M (2012) Innate and adaptive antifungal immune responses: partners on an equal footing. Mycoses 55:205–217
Hamilton TA, Becton DL, Somers SD (1985) Interferon-gamma modulates protein kinase C activity in murine peritoneal macrophages. J Biol Chem 260:1378–1381
Hao N, Lu M, Fan Y et al (2012) Macrophages in tumor microenvironments and the progression of tumors. Clin Dev Immunol 2012:11, 948098
Hernández-Santos N, Gaffen SL (2012) Th17 cells in immunity to *Candida albicans*. Cell Host Microbe 11(5):425–435
Hise AG, Tomalka J, Ganesan S et al (2009) An essential role for the NLRP3 inflammasome in host defense against the human fungal pathogen *Candida albicans*. Cell Host Microbe 5: 487–497
Huang G, Wang Y, Chi H (2012) Regulation of TH17 cell differentiation by innate immune signals. Cell Mol Immunol 9(4):287–295
Ito M, Yanagi Y, Ichinohe T (2012) Encephalomyocarditis virus viroporin 2B activates NLRP3 inflammasome. PLoS Pathog 8(8):1002857
Kanetsuma F, Carbonell LM (1970) Cell wall glucans of the yeast and mycelial forms of *Paracoccidioides brasiliensis*. J Bacteriol 101:675–680
Kanneganti TD, Ozoren N, Body-Malapel M et al (2007) Bacterial RNA and small antiviral compounds activate caspase-1 through cryopyrin/Nalp3. Nature 440:223–236
Kauffmann CA (1999) Sporotrichosis. Clin Infect Dis 29:231–236
Kazanas N (1986) Foodborne *Sporothrix schenckii*: infectivity for mice by intraperitoneal and intragastric inoculation with conidia. Mycopathologia 95:3–16
Kolb JP, Paul-Eugene N, Damais C et al (1994) Interleukin-4 stimulates cGMP production by IFN-gamma-activated human monocytes. Involvement of the nitric oxide synthase pathway. J Biol Chem 269:9811–9816

Korn T, Bettelli E, Oukka M et al (2009) IL-17 and Th17 Cells. Annu Rev Immunol 27:485–517
Kozel TR (1995) Virulence factors of *Cryptococcus neoformans*. Trends Microbiol 3:295–299
Kroemer G, Galluzzi L, Vandenabeele P et al (2009) Nomenclature Committee on Cell Death 2009. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. Cell Death Differ 16(1):3–11
Kumar H, Kawai T, Akira S (2011) Pathogen recognition by the innate immune system. Int Rev Immunol 30:16–34
Labbé K, Saleh M (2008) Cell death in the host response to infection. Cell Death Differ 15: 1339–1349
Lamkanfi M, Dixit VM (2014) Mechanisms and functions of inflammasomes. Cell 157:1013–1022
Laskin JD, Heck DE, Laskin DL (1994) Multifunctional role of nitric oxide in inflammation. Trends Endocrinol Metab 5:377–382
Laubach VE, Shesely EG, Smithies O (1995) Mice lacking inducible nitric oxide synthase are not resistant to lipopolysaccharide-induced death. Proc Natl Acad Sci U S A 92:10688–10692
Lee M, Yea SS (2000) Hydrogen peroxide inhibits the immune response to lipopolysaccharide by attenuating signaling through c-Jun N-terminal kinase and p38 associated with protein kinase C. Immunopharmacology 48:165–172
Lei PC, Yoshiike T, Yaguchi H et al (1993) Histopathological studies of *Sporothrix schenckii*-inoculated mice. Possible functions of polymorphonuclear leukocytes in normal and immunocompromised (congenitally athymic nude) mice. Mycopathologia 122(2):89–93
Lima-Junior DS, Costa DL, Carregaro V et al (2013) Inflammasome-derived IL-1β production induces nitric oxide-mediated resistance to *Leishmania*. Nat Med 19:909–915
Lurie HI (1971) Sporotrichosis. Human infection with fungi actinomycetes and algae. In: Handbuch der Speziellen Pathologischen. Anatomie und Histologie. RD Baker, Berlin, pp 614–675
Maia DCG, Sassá MF, Placeres MCP (2006) Influence of Th1/Th2 cytokines and nitric oxide murine systemic infection induced by *Sporothrix schenckii*. Mycopathologia 161:11–19
Malireddi RK, Kanneganti TD (2013) Role of type I interferons in inflammasome activation, cell death, and disease during microbial infection. Front Cell Infect Microbiol 3:77
Mantovani A, Sica A, Sozzani S et al (2004) The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 25:677–686
Mao L, Zhang L, Li H (2014) Pathogenic fungus *Microsporum canis* activates the NLRP3 inflammasome. Infect Immun 82:882–892
Martinez FO, Helming L, Gordon S (2009) Alternative activation of macrophages: an immunologic functional perspective. Annu Rev Immunol 27:451–483
McKenzie AN, Spits H, Eberl G (2014) Innate lymphoid cells in inflammation and immunity. Immunity 41(3):366–374
Mencacci A, Torosantucci A, Spaccapelo R et al (1994) A mannoprotein constituent of *Candida albicans* that elicits different levels of delayed-type hypersensitivity, cytokine production, and anticandidal protection in mice. Infect Immun 62:5353–5360
Mencacci A, Cenci E, Bacci A et al (2000) Cytokines in candidiasis and aspergillosis. Curr Pharm Biotechnol 1:235–251
Miranda LH, Conceição-Silva F, Quintella LP et al (2013) Feline sporotrichosis: histopathological profile of cutaneous lesions and their correlation with clinical presentation. Comp Immunol Microbiol Infect Dis 36(4):425–432
Monari C, Pericolini E, Bistoni G et al (2005) *Cryptococcus neoformans* capsular glucuronoxylomannan induces expression of Fas ligand in macrophages. J Immunol 174:3461–3468
Moreira JAS, Freitas DFS, Lamas CC (2015) The impact of sporotrichosis in HIV-infected patients: a systematic review. Infection 43(3):267–276
Moretti J, Blander JM (2014) Insights into phagocytosis-coupled activation of pattern recognition receptors and inflammasomes. Curr Opin Immunol 26:100–110

Munder M, Mallo M, Eichmann K et al (1998) Murine macrophages secrete interferon γ upon combined stimulation with interleukin (IL)-12 and IL-18: a novel pathway of autocrine macrophage activation. J Exp Med 187:2103–2108

Nascimento RC, Almeida SR (2005) Humoral immune response against soluble and fractionate antigens in experimental sporotrichosis. FEMS Immunol Med Microbiol 43:241–247

Navarre WW, Zychlinsky A (2000) Pathogen-induced apoptosis of macrophages: a common end for different pathogenic strategies. Cell Microbiol 2(4):265–273

Negrini TC, Ferreira LS, Arthur RA et al (2013) Role of TLR-2 and fungal surface antigens on innate immune response against *Sporothrix schenckii*. Immunol Invest 42:36–48

Ohteki T, Fukao T, Suzue K (1999) Interleukin-12-dependent interferon γ production by CD8α + lymphoid dendritic cells. J Exp Med 189:1981–1986

Okuda Y, Sakoda S, Shimaoka M, Yanagihara T (1996) Nitric oxide induces apoptosis in mouse splenic T lymphocytes. Immunol Lett 52:135–138

Paiva-Oliveira EL, Silva AC, da Silva RM et al (2012) Inflammasome and its clinical impact: literature review. Revista de Ciências Médicas e Biológicas 11:96–102

Palladino MA, Bahjat FR, Theodorakis EA et al (2003) Anti TNF-α therapies: the next generation. Nat Rev Drug Disc 2:736–746

Parslow TG, Bainton DF (1997) Innate immunity. In: Medical immunology. Appleton & Lange, Stamford, CT, pp 25–42

Philpott DJ, Yamaoka S, Israel A (2000) Invasive *Shigella flexneri* activates NF-Kappa B through a lipopolysaccharide – dependent innate intracellular response and leads to IL-8 expression in epithelial cells. J Immunol 165:903–914

Pick E, Keisari Y (1980) A simple colorimetric method for measurement of hydrogen peroxide by cells in culture. J Immunol Methods 38:61–70

Pierini R, Perret M, Djebali S, Juruj C et al (2014) ASC controls IFN-γ levels in an IL-18-dependent manner in caspase-1-deficient mice infected with *Francisella novicida*. J Immunol 191:3847–3857

Pluddemann A, Mukhopadhyay S, Gordon S (2011) Innate immunity to intracellular pathogens: macrophage receptors and responses to microbial entry. Immunol Rev 240:11–24

Puddu P, Fantuzzi L, Borghi P et al (1997) IL-12 induces IFN-γ expression and secretion in mouse peritoneal macrophages. J Immunol 159:3490–3497

Rajan JV, Rodriquez D, Miao EA et al (2011) The NLRP3 inflammasome detects encephalomyocarditis virus and vesicular stomatitis virus infection. J Virol 85:4167–4172

Ramasarma T (1990) H_2O_2 has a role in cellular regulation. Indian J Biochem Biophys 27: 269–274

Robinson BW, Mclemore TL, Crystal RG (1985) Gamma interferon is spontaneously released by alveolar macrophages and lung T lymphocytes in patients with pulmonary sarcoidosis. J Clin Invest 75:1488–1495

Romani L (2011) Immunity to fungal infections. Nat Rev Immunol 11(4):275–288

Said-Sadier N, Padilla E, Langsley G et al (2010) *Aspergillus fumigatus* stimulates the NLRP3 inflammasome through a pathway requiring ROS production and the Syk tyrosine kinase. PLoS One 5(4):e10008

Sargi SCMMO, Dalalio JV, Visentainer RC et al (2012) Production of TNF-α, nitric oxide and hydrogen peroxide by macrophages from mice with paracoccidioidomycosis that were fed a linseed oil-enriched diet. Mem Inst Oswaldo Cruz 107(3):303–309

Sasada M, Jonhston RB (1980) Macrophage microbicidal activity: correlation between phagocytosis-associated oxidative metabolism and killing of *Candida* by macrophages. J Exp Med 152:85–98

Sasai M, Yamamoto M (2013) Pathogen recognition receptors: ligands and signaling pathways by Toll-like receptors. Int Rev Immunol 32(2):116–133

Sassá MF, Saturi AET, Ferreira LS et al (2009) Response of macrophages Toll-like receptor 4 to a *Sporothirx schenckii* lipid extract during experimental sporotrichosis. Immunology (Oxford Print) 128:301–309

Sassá MF, Ribeiro LCA, Ferreira LS et al (2012) Immune response against *Sporothrix schenckii* in TLR-4-deficient mice. Mycopathologia 174:21–30

Schaffner A, Davis CE, Schaffner T et al (1986) In vitro susceptibility of fungi to killing by neutrophil granulocytes discriminates between primary pathogenicity and opportunism. J Clin Invest 78:511–524

Scott EN, Muchmore HG (1989) Immunoblot analysis of antibody responses to *Sporothrix schenckii*. J Clin Microbiol 27:300–304

Segal BH (2007) Role of macrophages in host defense against aspergillosis and strategies for immune augmentation. Oncologist 12:7–13

Sgarbi DB, da Silva AJ, Carlos IZ et al (1997) Isolation of ergosterol peroxide and its reversion to ergosterol in the pathogenic fungus *Sporothrix schenckii*. Mycopathologia 139:9–14

Shimada K, Crother TR, Karlin J et al (2011) Caspase-1 dependent IL-1β secretion is critical for host defense in a mouse model of *Chlamydia pneumoniae* lung infection. PLoS One 6(6)

Shimonaka H, Noguchi T, Kawai K et al (1975) 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 11:1187–1194

Siraishi A, Nakagaki K, Arai T (1992) Role of cell-mediated immunity in the resistance to experimental sporotrichosis in mice. Mycopathologia 120:15–21

Song DH, Lee JO (2012) Sensing of microbial molecular patterns by Toll-like receptors. Immunol Rev 250(1):216–229

Steele C, Wormley FL Jr (2012) Immunology of fungal infections: lessons learned from animal models. Curr Opin Microbiol 15(4):413–419

Strowig T, Henao-Mejia J, Elinav E et al (2012) Inflammasomes in health and disease. Nature 481:278–286

Svennerholm L, Fredman P (1980) A procedure for the quantitative isolation of brain gangliosides. Biochim Biophys Acta 17:97–109

Tachibana T, Matsuyama T, Mitsuyama M (1999) Involvement of CD4+ T cells and macrophages in acquired protection against infection with *Sporothrix schenckii* in mice. Med Mycol 37: 397–404

Takao S, Smith EH, Wang D et al (1996) The role of reactive oxygen metabolites in murine peritoneal macrophage phagocytosis and phagocytic killing. Am J Physiol 40:C1278–C1284

Tavares AH, Magalhães KG, Almeida RD et al (2013) NLRP3 inflammasome activation by *Paracoccidioides brasiliensis*. PLoS Negl Trop Dis 7:e2595

Taylor EL, Megson IL, Haslett C et al (2003) Nitric oxide: a key regulator of myeloid inflammatory cell apoptosis. Cell Death Differ 10(4):418–430

Uenotsuchi T, Takeuchi S et al (2006) Differential induction of Th1-prone immunity by human dendritic cells activated with *Sporothrix schenckii* of cutaneous and visceral origins to determine their different virulence. Int Immunol 18(12):1637–1646

Underhill DM, Ozinsky A (2002) Phagocytosis of microbes: complexity in action. Annu Rev Immunol 20:825–852

van de Veerdonk FL, Marijnissen RJ, Kullberg BJ et al (2009a) The macrophage mannose receptor induces IL-17 in response to *Candida albicans*. Cell Host Microbe 5:329–340

van de Veerdonk FL, Gresnigt MS, Kullberg BJ et al (2009b) Th17 responses and host defense against microorganisms: an overview. BMB Rep 42(12):776–787

van de Veerdonk FL, Netea MG, Dinarello CA et al (2011) Inflammasome activation and IL-1b and IL-18 processing during infection. Trends Immunol 32:110–116

van der Veen RC (2001) Nitric oxide and T helper cell immunity. Int Immunopharmacol 1: 1491–1500

Vazquez MI, Catalan-Dibene J, Zlotnik A (2015) B cells responses and cytokine production are regulated by their immune microenvironment. Cytokine. doi:10.1016/j.cyto.2015.02.007

Vera-Cabrera L, Salinas-Carmona MC, Waksman N et al (2012) Host defenses in subcutaneous mycoses. Clin Dermatol 30(4):382–388

Verdan FF, Faleiros JC, Ferreira LS et al (2012) Dendritic cell are able to differentially recognize *Sporothrix schenckii* antigens and promote Th1/Th17 response in vitro. Immunobiology 8: 788–794

Villena SN, Pinheiro RO, Pinheiro CS et al (2008) Capsular polysaccharides galactoxylomannan and glicuronoxylomannan from *Cryptococcus neoformans* induce macrophage apoptosis mediated by Fas ligand. Cell Microbiol 10(6):1274–1285

Voll RE, Herrmann M, Roth EA et al (1997) Immunosuppressive effects of apoptotic cells. Nature 390:350–351

Wellington M, Koselny K, Sutterwala FS et al (2014) *Candida albicans* triggers NLRP3-mediated pyroptosis in macrophages. Eukaryot Cell 13:329–340

Wu-Hsieh BA, Chen W, Lee H-J (1998) Nitric oxide synthase expression in macrophages of *Histoplasma capsulatum*-infected mice is associated with splenocyte apoptosis and unresponsiveness. Infect Immun 68(11):5520–5526

Yang CS, Shin DM, Jo EK et al (2012) The role of NLR-related protein 3 inflammasome in host defense and inflammatory diseases. Int Neurourol J 16:2–12

Zeng W, Wang X, Xu P et al (2015) Molecular imaging of apoptosis: from micro to macro. Theranostics 20:559–582

Zhu J, Yamane H, Paul WE (2010) Differentiation of effector CD4 T cell populations. Annu Rev Immunol 28:445–489

Zumsteg U, Frigerio S, Holländer GA (2000) Nitric oxide production and Fas surface expression mediate two independent pathways of cytokine-induced murine β-cell damage. Diabetes 49: 39–47

Chapter 8
Diagnosis of Sporotrichosis: Current Status and Perspectives

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Abstract Definitive diagnosis of sporotrichosis is based on fungal detection in culture. Microscopic methods for the detection of *Sporothrix* yeast cells in clinical samples have low sensitivity. Although culture methods have high sensitivity, they also have some limitations, such as the time required to conclude the diagnosis, usually from 10 to 15 days, and the difficulty of obtaining an adequate clinical specimen for the test in cases of extracutaneous sporotrichosis. Serological methods are useful tools for a presumptive diagnosis of this infection. The most-used antigenic *Sporothrix* molecules are the peptide-rhamnomannan and secreted exoantigens. The enzyme-linked immunosorbant assay (ELISA) technique using the peptide-rhamnomannan has high efficiency, and it is useful in the serological follow-up of infection. Exoantigens were first used in immunoprecipitation and agglutination tests, but they have been used more recently in immunoenzymatic tests, with high sensitivity and specificity for both human and feline disease. A glycoprotein of 70 kDa was purified from *Sporothrix* exoantigens, presenting high immunogenicity, which allows its use in the development of more sensitive and specific methods for sporotrichosis serodiagnosis. Molecular methods of diagnosis can lower the time for diagnosis conclusion, but described methodologies in this field are scarce. In conclusion, the diagnosis of sporotrichosis is a challenging field, and the development of new serological and molecular diagnostic methods is mandatory.

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8.1 Introduction

Mycoses can be challenging to diagnose, and accurate interpretation of laboratory data is important to ensure appropriate treatment. Although the clinical manifestations of sporotrichosis are well described, the diagnosis of this mycosis cannot be based on clinical information alone because the symptoms of sporotrichosis overlap with those of other diseases.

Sporotrichosis is classically diagnosed by correlating clinical, epidemiological, and laboratory data (Zancope-Oliveira et al. 2011). Typical laboratory analyses include microscopic examination using 10 % potassium hydroxide or 4 % sodium hydroxide to detect parasitic cigar-shaped, budding, yeast-like cells. These fungal cells are small (2–6 μm in diameter), rare, and difficult to detect during direct examination of specimens obtained from human patients (Kwon-Chung and Bennett 1992) or from domestic animals, such as dogs (Schubach et al. 2006). On the other hand, when this test is performed on skin biopsies collected from cats infected with *Sporothrix schenckii*, yeast cells are easily observed because cats have a high fungal burden on their lesions (Schubach et al. 2004). Microscopic methods for yeast detection in clinical samples are of low sensitivity (Barros et al. 2011), and asteroid body observation varies among different works (Quintella et al. 2011). However, definitive diagnosis of sporotrichosis is based on fungal detection in culture (Zhang et al. 2011). Cultivation of clinical specimens in mycological media such as Sabouraud dextrose agar or mycobiotic agar yields white filamentous colonies that become brown to black after a few days. Subculturing these colonies in brain–heart infusion at 35–37 °C results in white to creamy yeast-like colonies (Barros et al. 2011). *S. schenckii* identification is based on the macro- and micromorphologies of the mycelial (Fig. 8.1) and yeast forms (Zancope-Oliveira et al. 2011). However, these characteristics do not differentiate the newly described species of the *Sporothrix* complex. To physiologically differentiate the species within this complex, other tests such as carbohydrate assimilation (especially sucrose and raffinose), growth rates at 30 and 37 °C, as well as production of dematiaceous sessile conidia are necessary (Marimon et al. 2007, 2008). However, discrepancies between physiological and molecular methods of identification have been described (Oliveira et al. 2011). Moreover, although positive cultures provide the strongest evidence for sporotrichosis, there are some significant limitations. In particular, in some manifestations of the disease, such as *S. schenckii*–induced arthritis, the collection of material for culture is difficult (Morris-Jones 2002). In addition, sporotrichosis may be mistaken for other infections, such as tuberculosis, leishmaniasis, paracoccidioidomycosis, gummatous syphilis, and chromoblastomycosis (Rippon 1988; Sharma et al. 2005).

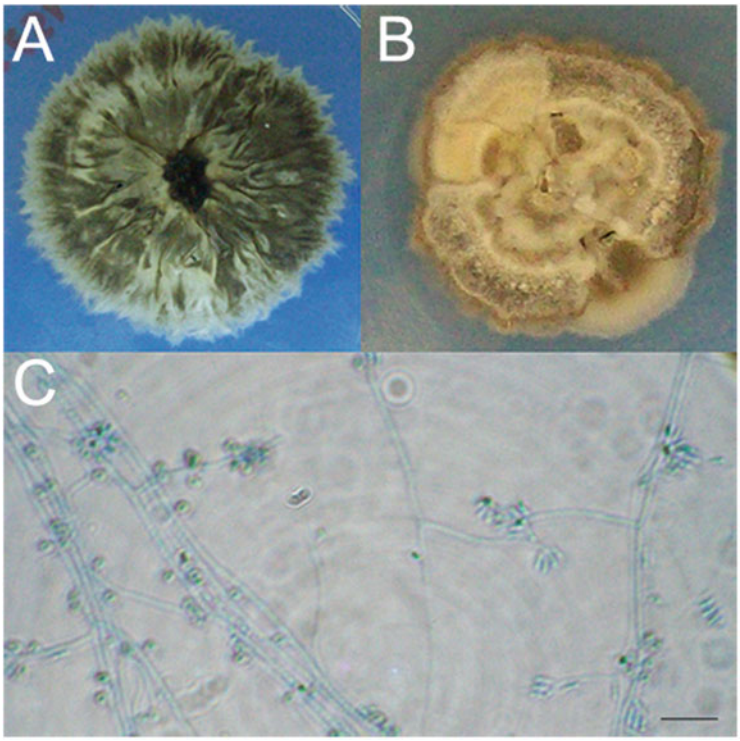

Fig. 8.1 Morphologic characteristics of the mycelial form of the *Sporothrix* complex. (**a**) *Sporothrix schenckii* sensu stricto colony on potato dextrose agar, incubated at 30 °C for 21 days. (**b**) Colony of a *S. brasiliensis* strain, showing a similar morphology to the *S. schenckii* strain. (**c**) Slide culture of the *S. schenckii* strain, showing hyaline conidia on sympodial conidiophores and dematiaceous conidia on denticles arising from the hyphae. Bar 10 μm

Non-culture methods have been developed to improve the rate and speed of diagnosis. Additional diagnostic tools are currently available for diagnosis of sporotrichosis to supplement culture and microscopic examination. These laboratory tests have a rapid turnaround time and reasonable specificity and sensitivity. For instance, serological techniques involving antibody detection have been developed using different methodologies. Molecular methods to detect *Sporothrix* species complex DNA in clinical specimens, including tissue fragments, are also being studied in several laboratories to facilitate rapid diagnosis of infection (Ruiz-Baca et al. 2013; Oliveira et al. 2014). The results from the described tests can provide a presumptive diagnosis of sporotrichosis and require clinical correlation for the correct evaluation and determination of the final diagnosis.

Serological techniques are usually simpler than culture and very useful in the diagnosis and follow-up of patients with sporotrichosis. The following serologic tests have been applied in the diagnosis of Diagnostic methods: agar gel immunodiffusion (ID), slide (SLA), tube (TA) and latex agglutination (LA), complement

fixation reaction (CF), immunofluorescence (IF) (Blumer et al. 1973), immunoelectrophoresis (IEP) (Albornoz et al 1984), and immunoenzyme assays in several formats, such as ELISAs, and immunoblots. These techniques have advanced considerably in recent decades because of the development of innovative detection schemes in the identification of relevant *Sporothrix* spp. antigens. However, the literature concerning the serodiagnosis of sporotrichosis is neither extensive nor diverse.

A range of antigenic preparations, derived both from whole yeast cells and from culture filtrate in their crude and/or purified states, have been applied in serological tests, which has resulted in high cross-reactivity, one of the most persistent problems found in the serodiagnosis of sporotrichosis. Moreover, these antigenic preparations are highly variable, making it very difficult to standardize diagnostic techniques in different laboratories.

The next sections focus on the main antigens and serologic methods applied for the presumptive diagnosis of sporotrichosis, as well as the application of some antigenic preparations in skin tests and molecular diagnosis.

8.2 Antigen Detection

The antigenic composition of the members of the *Sporothrix* complex is poorly understood. The most studied molecules with immunological reactivity of these fungi are the peptide-rhamnomannan, the exoantigens, and the newly described gp-70.

The *Sporothrix* peptide-rhamnomannan is a fraction of the fungal cell wall that presents an affinity to concanavalin A(Con-A) and that reacts with antibodies present in sera from patients with sporotrichosis (Lloyd and Bitoon 1971). Through a western blot analysis, this Con-A binding fraction of the *Sporothrix* cell wall was characterized as a mixture of three antigenic fractions of 84, 70, and 58 kDa. Moreover, through a β-elimination procedure, it was verified that the 70-kDa molecule is a deglycosylated form of the 84-kDa antigen (Lopes-Bezerra and Lima 1997). This antigen is stable, and its immunological reactivity does not significantly change if different *Sporothrix* strains are used in the extract preparation (Bernardes-Engemann et al. 2009). The potential application of this antigen to the serodiagnosis of sporotrichosis was initially suggested by the observation that cross-reactions with paracoccidioidomycosis, cryptococcosis, aspergillosis, candidiasis, and histoplasmosis were absent in the Con-A binding fraction of the cell wall peptide-rhamnomannan (Loureiro Y Penha and Lopes-Bezerra 2000). Later, an ELISA to detect immunoglobulin (Ig)-G in serum from patients with several clinical forms of sporotrichosis was described using this specific antigenic fraction, with a sensitivity of 90 % and specificity of 80 % (Bernardes-Engemann et al. 2005). The Con-A binding fraction of the *Sporothrix* cell wall peptide-rhamnomannan can also be used to detect antibodies in the synovial fluid (Costa

et al. 2008) and to monitor therapeutic response to antifungal treatment (Orofino-Costa et al. 2009).

The exoantigens from *Sporothrix* spp. were first applied in several immunological assays, such as IEP, LA, and double ID (Karlin and Nielsen 1970; Blumer et al. 1973; Casserone et al. 1983; Albornoz et al. 1984). However, there were no standard procedures for the production of the antigenic extracts used. In fact, changes in sugar composition of the exoantigens occur during *Sporothrix* growth, indicating a need for standardization (Takata and Ishizaki 1983). Differences in exoantigen composition and immunological reactivity were also related to the morphological state of the fungus (Albornoz et al. 1984), to the culture medium employed (Mendoza et al. 2002; Fernandes et al. 2009), and to the geographical origin of strains (Fernandes et al. 2009). Differences in exoantigen composition among the species of the *Sporothrix* complex are poorly studied, but it does not appear to significantly impact the antigenic composition of secreted molecules. A recent study showed heterogeneous protein profiles of exoantigens obtained from the mycelial form of *S. brasiliensis*, *S. globosa*, and *S. schenckii sensu stricto* in Sabouraud dextrose medium. The proteins of 60 and 46 kDa were observed in all extracts, regardless of species. Moreover, it was not possible to characterize specific secreted molecules for these species (Fernandes et al. 2013). Similar observations were obtained with the secreted antigens from the yeast form of *S. brasiliensis* and *S. schenckii s. str*. A molecule of 85 kDa was detected in both species, and no significant differences in antigenic composition or immunological reactivity were observed among the species (Almeida-Paes et al. 2012).

In a study of the yeast-phase *Sporothrix* exoantigens, it was verified that a 70-kDa antigenic fraction was always reactive against serum antibodies present in experimentally infected mice after 14 days of infection (Nascimento and Almeida 2005). Further studies against this antigen showed that a monoclonal antibody with affinity to this protein was able to enhance phagocytosis by macrophages. Moreover, this monoclonal antibody reduced the fungal burden and inhibited the *Sporothrix* interaction with the extracellular matrix (Nascimento et al. 2008). The role of this antigen as an adhesin was elucidated in subsequent studies (Ruiz-Baca et al. 2009; Teixeira et al. 2009). It is interesting to note that an immunological reactivity against the *Sporothrix* gp-70 was consistently observed in these studies, which encourages its use in the serodiagnosis of sporotrichosis. Furthermore, this antigen is produced by the three major species of the *Sporothrix* complex (*S. brasiliensis*, *S. globosa*, and *S. schenckii*), thus allowing the sporotrichosis diagnosis regardless of the infective species (Ruiz-Baca et al. 2014).

In general, serological tests as an aid for diagnosis do not use purified or recombinant antigens, because described immune reactive proteins are scarce, especially for the newly described *Sporothrix* species such as *S. brasiliensis*. An immunoblot assay allied with computer-based analysis was used to identify putative antigenic molecules in cell-free extracts of both morphological phases of this fungus, and to delineate antigenic polymorphism among seven *S. brasiliensis* isolates and one *S. schenckii* Brazilian strain. The mycelial and yeast phase of the fungus originated 14 and 23 reactive bands, respectively, which varied in intensity.

An 85-kDa antigen, verified in the yeast phase of the fungus, was observed in all strains used, and the immunodominant protein was identified. However, this protein cross-reacted with sera samples from patients infected with other pathogens (Almeida-Paes et al. 2012). It was also demonstrated that the use of different strains or even the morphological form of *Sporothrix* isolates could have an effect on the antigenic reactivity of a *Sporothrix* extract. Therefore, an adequate standardization of antigens must be produced before their general use in the serodiagnosis of sporotrichosis.

8.3 Antibody Detection

8.3.1 Immunoprecipitation and Agglutination Techniques

Immunoprecipitation and agglutination methodologies were first used in the diagnosis of sporotrichosis in the period 1970–1980 (Albornoz et al. 1984; Blumer et al. 1973; Casserone et al. 1983; Karlin and Nielsen 1970). The first TA and CF tests for sporotrichosis were reported in 1910 (Widal et al. 1910), and a diagnostic precipitin test that employed a polysaccharide antigen was subsequently described by González-Ochoa and Figueroa (1947). The ID test for sporotrichosis usually does not cross-react with sera from patients with chromoblastomycosis or leishmaniasis, infectious diseases with similar clinical manifestations (Albornoz et al. 1984). IEP has also been used, and in all positive cases, an anodic arc, called an S arc, is observed (Albornoz et al. 1984). Both methodologies that use an antigenic complex from fungal culture filtrate are highly sensitive. TA and LA both have high sensitivity and specificity and have been used for sporotrichosis serodiagnosis since the 1970s (Blumer et al. 1973; Casserone et al. 1983; Karlin and Nielsen 1970). However, these tests lack sensitivity in cases of cutaneous sporotrichosis (Albornoz et al. 1984; Rippon 1988) and do not enable the determination of the immunoglobulin isotype involved. The lack of standardization of reagents and methodologies applied in these techniques means they are not routinely used in the diagnosis of sporotrichosis in clinical laboratories.

8.3.2 Immunoenzymatic Assays

The serodiagnosis of this mycosis has increasingly used immunoassays. The first immunoblot assay used for diagnosis of sporotrichosis dates back to 1989, when exoantigen preparations from the *S. schenckii* yeast form showed 100 % sensitivity and 95 % specificity for the detection of antibodies (Scott and Muchmore 1989). Later, another immunoassay (ELISA) was developed, using the Con-A binding peptide-rhamnomannan from the *S. schenckii* yeast cell wall, and antibodies were

detected in 35 serum samples from patients with culture-proven sporotrichosis, resulting in 100 % sensitivity. However, the specificity was lower than previous tests because there was cross-reactivity with sera from patients with cutaneous leishmaniasis (Penha and Lopes-Bezerra 2000). The same group reported on an ELISA test using the same antigenic preparation against sera from 92 patients with different clinical forms of sporotrichosis in Rio de Janeiro and reported 90 % sensitivity, 80 % specificity, and a global efficiency of 86 % (Bernardes-Engemann et al. 2005). Other studies showed that the use of different strains during the preparation of the antigen might result in different sensitivity and specificity, despite the purification of the antigen involved in this methodology. This difference is due to the *O*-glycan residues linked to the molecules (Bernardes-Engemann et al. 2009).

An ELISA to detect IgG antibodies reactive to the mycelial-phase *Sporothrix* exoantigens produced after 21 days of growth in Sabouraud dextrose medium at 28 °C showed 97 % sensitivity and 89 % specificity when performed on 90 sera from patients with different clinical forms of sporotrichosis, 72 sera from patients with other infectious diseases, and 76 healthy controls. The major antigenic components present in this preparation were proteins of 90, 70, 63, 51, and 42 kDa (Almeida-Paes et al. 2007a). This assay was further improved to detect IgG, IgM, and IgA antibodies, which improves the global efficiency for diagnosis and therapeutic follow-up of sporotrichosis (Almeida-Paes et al. 2007b). Mendoza et al. (2002) previously described this exoantigen, and the lack of cross-reactivity of this preparation with sera from patients with other mycoses was remarkable. The same antigen was used previously in ID and IEP techniques without cross-reactivity with sera from patients with leishmaniasis or chromoblastomycosis (Albornoz et al. 1984).

When the ELISAs probed with different antigenic preparations are compared, the crude exoantigens (Almeida-Paes et al. 2007a) gave slightly higher sensitivity and specificity than those using the con-A binding fraction of the *S. schenckii* yeast cell wall (Bernardes-Engemann et al. 2005). A similar observation was found when using this con-A binding fraction and crude exoantigens for the serodiagnosis of feline sporotrichosis. The use of crude exoantigens showed slightly better results than the purified peptide-rhamnomannan in terms of sensitivity (96 and 90 %) and specificity (98 and 96 %, respectively), suggesting that the *Sporothrix* secreted proteins are highly immunogenic and specific (Fernandes et al. 2011).

More recently, an immunoblot assay using cell-free exoantigens of the yeast form of *S. brasiliensis* was described to detect IgG antibodies in serum samples from human patients. In this assay, a sensitivity of 100 % was achieved, but a low specificity (50 %) was observed. However, if the authors verified a positive serum sample only if at least two immunological bands appeared in the immunoblots, the specificity increased to 80 % with 93 % sensitivity (Almeida-Paes et al. 2012).

8.3.3 Sporotrichin Skin Test

The skin test (ST), with mycelial extracts or yeast cells called sporotrichin, has been widely used throughout the world, especially for epidemiological studies of sporotrichosis. It has also been applied as support for diagnosis in Latin America (Lopes-Bezerra et al. 2006; Barros et al. 2011; Bonifaz and Vázquez-González 2013) and in atypical forms of the disease, such as the case of a recent bulbar conjunctival sporotrichosis (Kashima et al. 2010). However, sporotrichin is not available commercially in many countries (Dominguez-Soto and Hojyo-Tomoka 1983; Bonifaz and Vázquez-González 2013), but it is accessible in many institutions dedicated to biomedical research. Reports are contradictory concerning its usefulness as a diagnostic tool due to false-positive results without signs or symptoms of the disease, but this condition suggests a previous immunosensitizing contact (exposure) with *S. schenckii*. Other reports mention almost 100 % positive sporotrichin ST in lymphocutaneous and fixed cutaneous forms of sporotrichosis (Barros et al. 2011; Bonifaz and Vázquez-González 2010).

The use of sporotrichin ST in epidemiological surveys has been extensively developed from the first surveys in Mexico (González-Ochoa and Ricoy 1970), Guatemala (Mayorga et al. 1978), and Brazil (Rocha-Posada 1968), among others. This practical test has been used until the present (Bonifaz et al. 2013), demonstrating the usefulness of these antigens to identify endemic regions of sporotrichosis worldwide, an option to gain insight in disease emergence scenarios such as the recent cat zoonosis in Brazil (Barros et al. 2004; Oliveira et al. 2011; Freitas et al. 2010; Silva et al. 2012).

Concerning the term sporotrichin, there are reports of different type of antigens. Culture filtrate extracts from the mycelial (28 °C) or yeast (37 °C) forms, commonly called metabolic antigens, were first described by González-Ochoa and Figueroa (1947) and consisted of glycopeptide antigens. Another described and used antigen is a 1:1000, 1:2000, or 1:4000 dilution of heat-killed yeast cells. In addition, their biological properties depend on culture conditions (Takata and Ishizaki 1983; Arenas and Toriello 1986). A commonly used antigen for epidemiological surveys is a culture filtrate of the mycelial form standardized to 10 μg protein/0.1 ml of intradermal ST (Toriello et al. 1991; Bonifaz et al. 2013). The ST may be applied on the back or forearm and readings made at 24 and 48 h. A reaction of 8 mm of induration after 24 and 48 h constitutes a positive test.

The lack of standardization in the above-mentioned antigens would account for the difference in reactivity with sporotrichin ST, such as a 6.25 % positivity with a 1:4000 dilution of a yeast antigen in the southern state of Oaxaca, Mexico with *S. schenckii* isolates recovered from soil (Sánchez-Aleman et al. 2004). This is in contrast with the 14 % positivity observed with a mycelial metabolic antigen at 10 μg protein/0.1 ml in an endemic region of sporotrichosis in Puebla, Mexico, without any fungal isolation (Mendez-Tovar et al. 2003).

Additional epidemiological studies of sporotrichosis and the *S. schenckii* complex are necessary because changes in the interplay of pathogens, hosts, and

environment lead to the formation of novel disease patterns as observed for sporotrichosis.

8.4 Molecular Diagnosis

Molecular methods have been developed based on polymerase chain reaction (PCR) techniques that show good sensitivity, specificity, and speed for early diagnosis of *S. schenckii* (Berbee and Taylor 1992; Kano et al. 2001, 2003; Hu et al. 2003; Xu et al. 2010; Mendoza et al. 2012; Liu et al. 2013; Ruiz-Baca et al. 2013; Oliveira et al. 2014). Sandhu et al. (1995) proposed the pioneering DNA-based methodologies used for the diagnosis of fungal infections, and developed 21 specific nucleic acid probes targeting the large subunit of the ribosomal RNA (*rRNA*) gene from several fungi, including *S. schenckii*. Among the major genes described for the diagnosis of sporotrichosis are the chitin-synthase gene 1 (*CHS*1), 18S rDNA, and mitochondrial DNA (mtDNA) (Berbee and Taylor 1992; Kano et al. 2001, 2003; Hu et al. 2003; Xu et al. 2010). The trials with nested PCR using the 18S rDNA gene and mtDNA as targets showed high sensitivity and specificity, indicating that these tests can provide a rapid diagnosis with sufficient accuracy to be used clinically in patients with sporotrichosis (Hu et al. 2003; Xu et al. 2010; Oliveira et al. 2014). A drawback of these methods is that they are not commercially available and require specialized equipment; therefore, they are not carried out in most clinical laboratories.

8.5 Conclusions and Perspectives

Sporotrichosis is classically diagnosed by correlation among clinical, epidemiological, and laboratory data. The conventional method for definitive diagnosis of sporotrichosis is based on etiological agent isolation in culture and its identification. A disadvantage of the culture methods, is that they are difficult to apply in disseminated and/or systemic sporotrichosis; therefore, detection of an antibody response in patients could provide a faster method for diagnosis. Sporotrichin ST remains an option for epidemiological studies to gain insight in disease emergence scenarios such as the recent cat zoonosis in Brazil. To date, serological techniques (like ELISA, Western blot, immunodiffusion, etc.) are used for sporotrichosis diagnosis and involve antibody detection against soluble antigens and different molecules of the *S. schenckii* cell wall, such as peptide-rhamnomannans, exoantigens, and the newly described molecule gp70. The sensitivity and specificity of these methods differ depending on the antigenic fraction used. Fluorescent antibodies and immunohistochemical techniques are alternative methods that also provide a rapid diagnosis of sporotrichosis in tissue samples. The diagnosis of *S. schenckii* by PCR in clinical samples has also shown a high degree of sensitivity

and specificity. These tests can provide a rapid diagnosis with sufficient precision to be used clinically for patients with sporotrichosis, with PCR studies for the disseminated cases of the disease.

The different methods described in this review are not available in many clinical laboratories, which compromises the ability to diagnose and provide individual treatment for sporotrichosis. The search for new protein biomarkers for the development of an easy, sensitive, and specific methodology could help lower the treatment costs and offer alternatives to current tests for the diagnosis of sporotrichoid infections that can be confused by infections caused by other pathogens. To date, efforts continue to improve or develop new diagnostic tests that are more sensitive and specific for this mycosis. However, these tests require validation prior to general application in routine diagnosis. The development and application of new biomarker molecules for the diagnosis of sporotrichosis remains to be done either with exoantigens or cell wall antigens. The recent release of the *S. schenckii* genome, and the use of certain tools such as genomics, proteomics, inmunoproteomics, metabolomics, interactomics, etc., would enable major advances in the search and identification of new biomarker antigens for sporotrichosis, which are expected to be introduced to the clinical laboratory in the short or medium term.

References

Albornoz MB, Villanueva E et al (1984) Application of immunoprecipitation techniques to the diagnosis of cutaneous and extracutaneous forms of sporotrichosis. Mycopathologia 85:177–183

Almeida-Paes R, Pimenta MA, Pizzini CV et al (2007a) Use of mycelial-phase *Sporothrix schenckii* exoantigens in an Enzyme-linked Immunosorbent Assay for diagnosis of sporotrichosis by antibody detection. Clin Vac Immunol 14:244–249

Almeida-Paes R, Pimenta MA, Monteiro PC et al (2007b) Immunoglobulins G, M, and A against *Sporothrix schenckii* exoantigens in patients with sporotrichosis before and during treatment with itraconazole. Clin Vac Immunol 14:1149–1157

Almeida-Paes R, Bailão AM, Pizzini CV et al (2012) Cell-free antigens of *Sporothrix brasiliensis*: antigenic diversity and application in an immunoblot assay. Mycoses 55:467–475

Arenas G, Toriello C (1986) Actividad inmunológica de antígenos miceliales y levaduriformes de diferentes fases de crecimiento de *Sporothrix schenckii*. Rev Mex Mic 2:131–144

Barros MB, Schubach AO, Valle AC et al (2004) Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: description of a series of cases. Clin Infect Dis 38:529–535

Barros MBL, Almeida-Paes R, Schubach AO (2011) *Sporothrix schenckii* and sporotrichosis. Clin Microbiol Rev 24:633–654

Berbee ML, Taylor JW (1992) 18S ribosomal RNA gene sequence characters place the human pathogen *Sporothrix schenckii* in the genus *Ophiostoma*. Exp Mycol 16:87–91

Bernardes-Engemann AR, Costa RC, Miguens BR et al (2005) Development of an enzyme-linked immunosorbent assay for the serodiagnosis of several clinical forms of sporotrichosis. Med Mycol 43:487–493

Bernardes-Engemann AR, Loureiro y Penha CV, Benvenuto F et al (2009) A comparative serological study of the SsCBF antigenic fraction isolated from three *Sporothrix schenckii* strains. Med Mycol 47:874–878

Blumer SO, Kaufman L, Kaplan W et al (1973) Comparative evaluation of five serological methods for the diagnosis of sporotrichosis. Appl Microbiol 26:4–8

Bonifaz A, Vázquez-González D (2010) Diagnostic methods: an update. G Ital Dermatol Venereol 145:659–673

Bonifaz A, Vázquez-González D (2013) Diagnosis and treatment of lymphocutaneous Diagnostic methods: what are the options? Curr Fungal Infect Rep 7:252–259

Bonifaz A, Araiza J, Perez-Mejía A et al (2013) Prueba intradérmica con esporotricina en una comunidad de la Sierra Norte de Puebla. Dermatol Rev Mex 57:428–432

Casserone S, Conti-Diaz IA, Zanetta E et al (1983) Serologia de laesporotricosis cutânea. Sabouraudia 21:317–321

Costa RO, de Mesquita KC, Damasco PS et al (2008) Infectious arthritis as the single manifestation of Diagnostic methods: serology from serum and synovial fluid samples as an aid to diagnosis. Rev Iberoam Micol 25:54–56

Dominguez-Soto L, Hojyo-Tomoka MT (1983) The intradermal sporotrichin test and the diagnosis of sporotrichosis. Int J Dermatol 22:520

Fernandes GF, Amaral CC, Sasaki A et al (2009) Heterogeneity of proteins expressed by *Sporothrix schenckii* isolates. Med Mycol 47:855–861

Fernandes GF, Lopes-Bezerra LM, Bernardes-Engemann AR et al (2011) Serodiagnosis of sporotrichosis infection in cats by enzyme-linked immunosorbent assay using a specific antigen, SsCBF, and crude exoantigens. Vet Microbiol 147:445–449

Fernandes GF, Santos PO, Rodrigues AM et al (2013) Characterization of virulence profile, protein secretion and immunogenicity of different *Sporothrix schenckii sensu stricto* isolates compared with *S. globosa* and *S. brasiliensis* species. Virulence 4:241–249

Freitas DFS, Valle ACF, Almeida-Paes R et al (2010) Zoonotic sporotrichosis in Rio de Janeiro, Brazil: a protracted epidemic yet to be curbed. Clin Infect Dis 50:453

González-Ochoa A, Figueroa ES (1947) Polisacaridos del *Sporotrichum schenckii*. Datos immunologicos: intradermoreaccion en el diagnostic de la esporotrichosis. Rev Inst Salubr Enferm Trop 8:143–153

González-Ochoa A, Ricoy E (1970) Valoración comparativa de los antígenos polisacáridos y celular de *Sporothrix schenckii*. Rev Invest Salud Publica 30:303–315

Hu S, Chung WH, Hung SI et al (2003) Detection of *Sporothrix schenckii* in clinical samples by a nested PCR assay. J Clin Microbiol 41:1414–1418

Kano R, Nakamura Y, Watanabe S et al (2001) Identification of *Sporothrix schenckii* based on sequences of the chitin synthase 1 gene. Mycoses 44:261–265

Kano R, Matsuoka A, Kashima M et al (2003) Detection of *Sporothrix schenckii* chitin synthase 1 (CHS1) gene in biopsy specimens from human patients with sporotrichosis. J Dermatol Sci 33:73–74

Karlin JV, Nielsen HS (1970) Serologic aspects of sporotrichosis. J Infect Dis 121:316–327

Kashima T, Honma R, Kishi S et al (2010) Bulbar conjunctival sporotrichosis presenting as a salmon-pink tumor. Cornea 29:573–576

Kwon-Chung KJ, Bennett JE (eds) (1992) Medical mycology. Lea & Febiger, London

Liu X, Zhang Z, Hou B et al (2013) Rapid identification of *Sporothrix schenckii* in biopsy tissue by PCR. J Eur Acad Dermatol Venereol 27:1491–1497

Lloyd KO, Bitoon MA (1971) Isolation and purification of a peptido-rhamnomannan from the yeast form of *Sporothrix schenckii*. Structural and immunochemical studies. J Immunol 107:663–671

Lopes-Bezerra LM, Lima OC (1997) Identification of a concavalin A-binding antigen of the cell surface of *Sporothrix schenckii*. J Med Vet Mycol 35:167–172

Lopes-Bezerra LM, Schubach A, Costa RO (2006) *Sporothrix schenckii* and sporotrichosis. Ann Acad Bras Ciencias 78:293–308

Marimon R, Cano J, Gené J et al (2007) *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. J Clin Microbiol 45:198–3206

Marimon R, Gené J, Cano J et al (2008) *Sporothrix luriei*: rare fungus from clinical origin. Med Mycol 46:621–625

Mayorga R, Cáceres A, Toriello C et al (1978) Etude d'une zone d'endemie sporotrichosique au Guatemala. Sabouraudia l6:l85–l98

Mendez-Tovar LJ, Lemini-López A, Hernández-Hernández F et al (2003) Frecuencia de micosis en tres comunidades de la sierra norte de Puebla. Gac Med Mex 139:1–6

Mendoza M, Diaz AM, Hung MB et al (2002) Production of culture filtrates of *Sporothrix schenckii* in diverse culture media. Med Mycol 40:447–454

Mendoza M, Brito A, Schaper DA et al (2012) Evaluación de la técnica PCR anidada para el diagnóstico de la esporotricosis experimental. Rev Iberoam Micol 29:120–125

Morris-Jones R (2002) Sporotrichosis. Clin Exp Dermatol 27:427–431

Nascimento RC, Almeida SR (2005) Humoral immune response against soluble and fractionate antigens in experimental sporotrichosis. FEMS Immunol Med Microbiol 43:241–247

Nascimento RC, Espíndola NM, Castro RA et al (2008) Passive immunization with monoclonal antibody against a 70-Kda putative adhesin of *Sporothrix schenckii* induces protection in murine sporotrichosis. Eur J Immunol 38:3080–3089

Oliveira MME, Almeida-Paes R, Muniz MM et al (2011) Phenotypic and molecular identification of *Sporothrix* isolates from an epidemic area of sporotrichosis in Brazil. Mycopathologia 172:257–267

Oliveira MME, Almeida-Paes R, Gutierrez-Galhardo MC et al (2014) Molecular identification of the *Sporothrix schenckii* complex. Rev Iberoam Micol 31:2–6

Orofino-Costa R, Bóia MN, Magalhães GAP et al (2009) Arthritis as a hypersensitivity reaction in a case of sporotrichosis transmitted by a cat: clinical and serological follow up of 13 months. Mycoses 53:81–83

Penha CV, Lopes-Bezerra LM (2000) Concanavalin A-binding cell wall antigens of *Sporothrix schenckii*: a serological study. Med Mycol 38:1–7

Quintella LP, Passos SRL, Valle ACF et al (2011) Histopathology of cutaneous sporotrichosis in Rio de Janeiro: a series of 119 consecutive cases. J Cutan Pathol 38:25–32

Rippon JW (1988) Sporotrichosis. Medical mycology: the pathogenic fungi and pathogenic actinomycetes. WB Saunders, Philadelphia

Rocha-Posada H (1968) Pruebacutánea con esporotricina. Mycopathologia 36:42–54

Ruiz-Baca E, Toriello C, Pérez-Torres A et al (2009) Isolation and some properties of a glycoprotein of 70 kDa (Gp70) from the cell wall of *Sporothrix schenckii* involved in fungal adherence to dermal extracellular matrix. Med Mycol 47:185–196

Ruiz-Baca E, Cuéllar-Cruz M, López-Romero E, Reyes Montes MR, Toriello C (2013) Fungal cell wall antigens for the diagnosis of invasive fungal infections. Fungal cell wall. Nova Science Publishers, Inc., New York, pp 207–208

Ruiz-Baca E, Hérnandez-Mendoza G, Cuéllar-Cruz M et al (2014) Detection of 2 immunoreactive antigens in the cell wall of *Sporothrix brasiliensis* and *Sporothrix globosa*. Diagn Microbiol Infect Dis 79:328–330

Sánchez-Aleman MA, Araiza J, Bonifaz A (2004) Aislamiento y caracterización de cepas silvestres de *Sporothrix schenckii* e investigación de reactores a la esporotricina. Gac Med Mex 140:507–513

Sandhu GS, Kline BC, Stockman L et al (1995) Molecular probes for diagnosis of fungal infections. J Clin Microbiol 33:2913–2919

Schubach TMP, Schubach AO, Okamoto T et al (2004) Evaluation of an epidemic of sporotrichosis in cats: 347 cases (1998-2001). J Am Vet Med Assoc 224:1623–1629

Schubach TMP, Schubach AO, Okamoto T et al (2006) Canine sporotrichosis in Rio de Janeiro, Brazil: clinical presentation, laboratory diagnosis and therapeutic response in 44 cases (1998-2003). Med Mycol 44:87–92

Scott EN, Muchmore HG (1989) Immunoblot analysis of antibody responses to *Sporothrix schenckii*. J Clin Microbiol 27:300–304

Sharma S, Choudhary R, Juneja M et al (2005) Cutaneous tuberculosis mimicking sporotrichosis. Indian J Pediatr 72:86

Silva MB, Costa MM, Torres CC et al (2012) Urban Diagnostic methods: a neglected epidemic in Rio de Janeiro, Brazil. Cad Saude Publica 28:1867–1880

Takata M, Ishizaki H (1983) Correlations among culture times, sugar composition and biological activities of *Sporothrix schenckii* antigens. Mycopathologia 84:31–39

Teixeira PAC, Castro RA, Nascimento RC et al (2009) Cell spots surface expression of adhesins for fibronectin correlates with virulence of *Sporothrix schenckii*. Microbiology 155:3730–3738

Toriello C, ArjonaRosado L, Taylor ML (1991) Efficiency of crude and purified fungal antigens in serodiagnosis to discriminate mycotic from other respiratory diseases. Mycoses 34:133–140

Widal F, Abrami P, Joltrain E et al (1910) Sero diagnostic mycosique. Applications audiagnostic de la sporotrichose et de l'ctinomycose. Les coagglutination set cofixations mycosiques. Ann Inst Pasteur 24:1–33

Xu TH, Lin JP, Gao XH et al (2010) Identification of *Sporothix schenckii* of various mtDNA types by nested PCR assay. Med Mycol 48:161–165

Zancope-Oliveira RM, Almeida-Paes R, Oliveira MME et al (2011) New diagnostic applications in sporotrichosis. In: Khopkar U (ed) Skin biopsy perspectives, 1st edn. InTech, Croatia

Zhang Z, Liu X, Lv X et al (2011) Variation in genotype and higher virulence of a strain of *Sporothrix schenckii* causing disseminated cutaneous sporotrichosis. Mycopathologia 172:439–446

Chapter 9
Therapeutic and Prophylactic Tools for Sporotrichosis: Current Strategies and Future Tendencies

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Abstract Over the past 3 decades, important progress in the therapy of fungal infections has been made. Although several chemotherapeutic agents are relatively effective against the different species of the *Sporothrix schenckii* complex, even the primary drugs used to treat sporotrichosis are the ergosterol inhibitors: triazole compounds, terbinafine, and amphotericin B. Despite the general effectiveness of these drugs, diverse problems remain, including chronic therapy, toxicity manifestations, and fungal resistance, which limit their use. These problems have stimulated a search for new agents that might be active against a wide range of clinical isolates, that are well absorbed after oral administration, that are widely distributed throughout body tissues, including the central nervous system, and that are relatively nontoxic. This chapter reviews the current therapy strategies for human and animal sporotrichosis and updates the more relevant strategies in study for prophylaxis and treatment.

Keywords *Sporothrix schenckii* • Sporotrichosis • Treatment • Antifungal drugs • Prophylaxis

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9.1 Introduction

Despite recent advances in antifungal therapy applied to the treatment of sporotrichosis, many unresolved aspects remain in terms of the long therapy regimen, fungal resistance, and toxicity. Although amphotericin B remained the drug of choice for the treatment of invasive fungal infections for several years, its use is limited by its serious toxic effects. Nephrotoxicity is the major adverse effect limiting the use of amphotericin B. The major manifestations of nephrotoxicity are decreased glomerular filtration, loss of urinary concentrating ability, renal loss of sodium and potassium, and renal tubular acidosis (Baginski and Czub 2009; Denning and Hope 2010).

Over the past 2 decades, the treatment of sporotrichosis has been improved with the introduction of new antifungal agents. The newer agents are less toxic and in some cases offer better efficacy than amphotericin B deoxycholate (ABD), which was the gold standard antifungal treatment for more than 40 years. The number of new antifungal drugs in development is growing; however, similar advances in treatments for sporotrichosis have not been seen because of the variable response and relative resistance among different clinical isolates of the *Sporothrix schenckii* complex. For this reason, much current research is being focused on drug delivery using different vehicles for the established antifungals, with the aim of improving efficacy and reducing toxicity. Other strategies include: novel antifungal combinations, the association of antifungals with immunomodulators, preventive approaches such as immunization, and the use of alternative therapies such as photodynamic therapy and medicinal plants.

9.2 Antifungal Drugs

Different classes of antifungal agents have been identified according to their mechanism of action (Fig. 9.1). The main antifungals used in the treatment of sporotrichosis are polyenes (amphotericin B); triazoles; and allylamines (terbinafine) (Fig. 9.2). The polyene antifungal antibiotics are isolated from various strains of *Actinomyces*. They are poorly soluble in water and common organic solvents, but they are reasonably soluble in highly polar solvents such as dimethyl sulfoxide and dimethylformamide. The main mechanism of action of polyene antifungals is binding to ergosterol in the phospholipid-sterol membranes of fungal cells to form complexes that induce the depolarization of the membrane, with a subsequent increase in membrane permeability followed by cell death (Zotchev 2003). Amphotericin B, which was introduced in the 1950s, remains the most

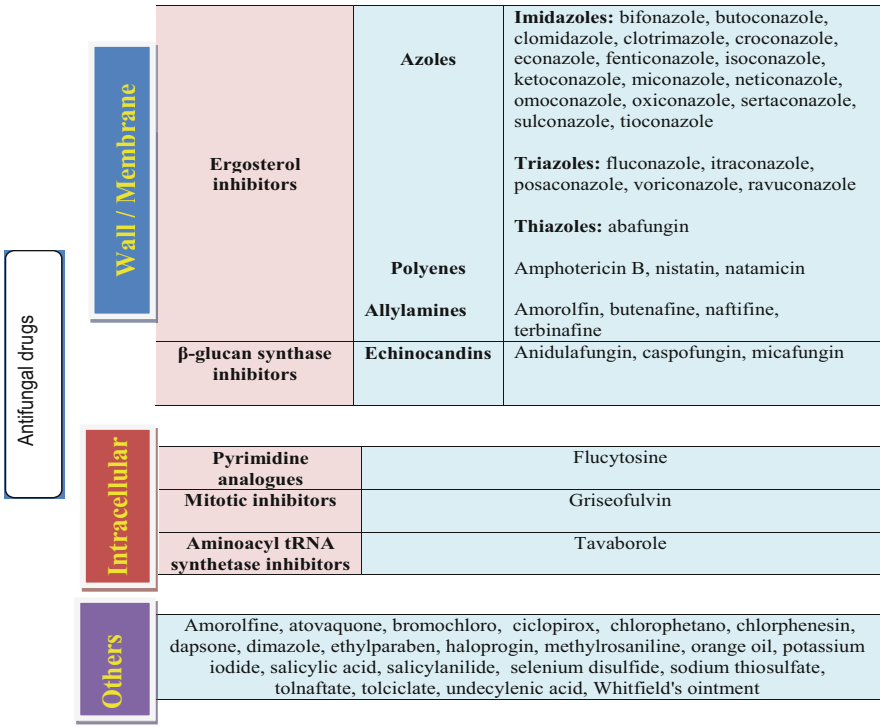

Fig. 9.1 Antifungal drugs and key mechanisms of action

valuable drug against the widest spectrum of human mycotic infections and is the most frequently used for disseminated mycoses (Denning and Hope 2010). The administration of amphotericin B can lead to a number of adverse effects, including anorexia, nausea, vomiting, fever, anemia, hepatic dysfunction, cardiac arrhythmias, allergic reactions, neurologic symptoms, and local thrombophlebitis. Thus amphotericin B is also available as liposomal- and lipid-based preparations to enhance the bioavailability of the drug and to reduce its toxicity without loss of efficacy (Torrado et al. 2008; Baginski and Czub 2009).

The azole antifungals are compounds that contain a nitrogen heterocyclic ring in their chemical structure. They include imidazoles (clotrimazole, miconazole, ketoconazole); triazoles (fluconazole, itraconazole, posaconazole, voriconazole, ravuconazole); and thiazoles (abafungin). Except for abafungin, which inhibits the enzyme sterol 24-C-methyltransferase (Borelli et al. 2008), azoles inhibit the enzyme sterol 14α-demethylase (CYP51), the enzyme that participates in the

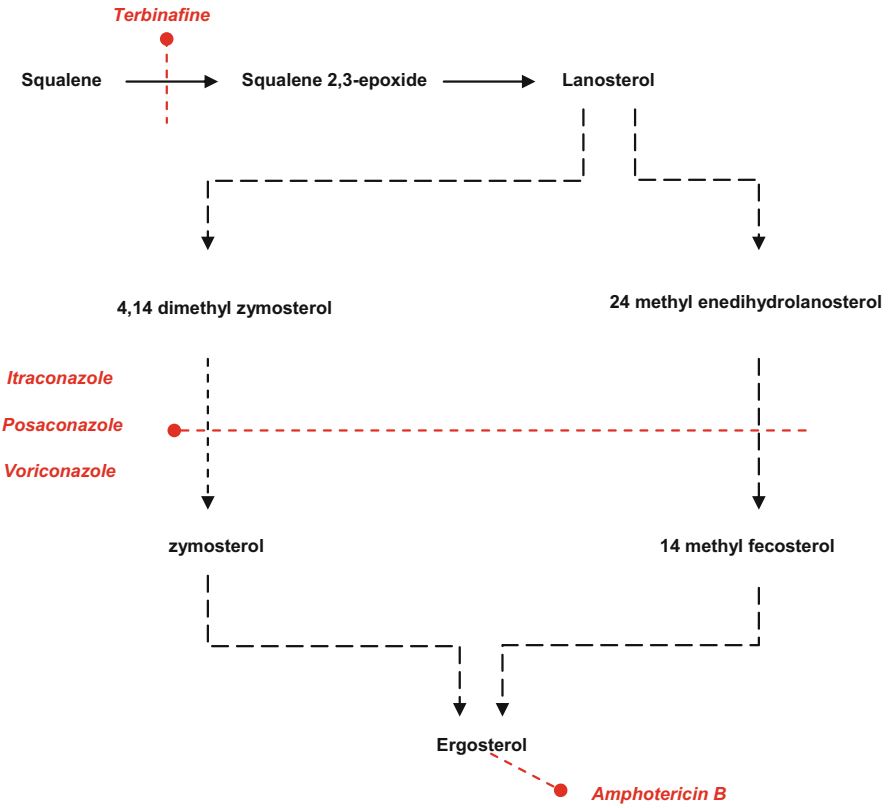

Fig. 9.2 Sites of action of the main antifungal agents used in the treatment of sporotrichosis

conversion of lanosterol to ergosterol in fungus. Depletion of ergosterol disrupts the structure and important functions of membrane, leading to fungal death (Warrilow et al. 2013).

As early as 1944, Woolley demonstrated inhibition of fungal growth with benzimidazole, an imidazole compound (Woolley 1944), but it was not until the 1970s that azole drugs were extensively evaluated for the treatment of systemic mycoses. The first oral azole proven to be effective in fungal infections was clotrimazole. However, treatment with clotrimazole led to the induction of liver microsomal enzymes, enhancing their own drug metabolism and thereby diminishing its antifungal activity (Saag and Dismukes 1988). Consequently, clotrimazole is now limited for topical or troche use (Czerninski et al. 2015). Newer triazoles (itraconazole, posaconazole, voriconazole, fluconazole) are now first-line drugs for the treatment of invasive fungal infections, including sporotrichosis (Kauffman et al. 2007; Laverdiere et al. 2014).

Terbinafine, like other allylamines, inhibits the squalene epoxidase, another enzyme important in ergosterol synthesis, causing fungal cell lysis (Nowosielski et al. 2011). Terbinafine has been successfully used in the treatment of many cases of sporotrichosis (Heidrich et al. 2011).

Beyond the suppression of fungal growth via a direct killing effect, other less explored but interesting mechanisms by which many of these drugs can act is via stimulation of the immune response with an indirect effect against the infection. The reported immunomodulator activities of these antifungal drugs include activation of the host's innate immunity effectors, such as the induction of oxidative metabolites, and proinflammatory cytokine production, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-8, thus enhancing the ability of the host to overcome mycotic infections (Mesa-Arango et al. 2012; Mihu et al. 2014). Several recent studies have revealed that many antifungal drugs are able to bind with receptors of innate immune cells, including toll-like receptors (TLRs) (Mihu et al. 2014) and NLRP3 inflammasome (Darisipudi et al. 2011), as a primary method of gaining access to dendritic cells and monocyte-macrophage system and activate.

The ability of antifungals to simultaneously elicit an efficient immune response is being researched for the potential development of new drugs to induce effective activation of the innate immune system as a way to synergistically help clear fungal infections (Mihu et al. 2014). Other strategies based on these findings of useful synergism between direct antifungal effect and immunomodulation is discussed later.

9.3 In Vitro Fungal Susceptibility

In vitro antifungal susceptibility testing is used to determine the sensitivity of a clinical fungal isolate to different antifungal drugs, to predict antifungal efficacy in the patient. In addition to its clinical use, susceptibility testing can also be used in both epidemiological studies and drug development as a screening tool to evaluate patterns of susceptibility or resistance of new substances against specific fungi compared with the well-established antifungals.

Initially, the concept of fungal minimum inhibitory concentration (MIC) testing was irrelevant, because no alternative to amphotericin B existed. With the introduction of other newer antifungal drugs, the growing numbers of opportunistic fungal pathogens, and the incidence of antifungal resistance, the concept of MIC testing became timely as a tool to select the most appropriate drug for therapy (Seibold and Tintelnot 2003).

In 1983, the National Committee for Clinical Laboratory Standards (NCCLS) established a subcommittee to standardize fungal MIC determination. Multiple groups of researchers worldwide were challenged to agree on standards that would generate reproducible MIC data in the range of normal serum drug concentrations and that would be sensitive enough to detect organisms with truly different

drug susceptibilities. As a result of this work, the NCCLS in 1997 adopted the M27-A protocol for the susceptibility testing of yeasts (NCCLS 1997). A second edition of that first guidance was published in 2002 (M27-A2) and a third edition in 2008 (M27-A3).

Despite the adoption and use of the M27 protocols by the NCCLS, some problems have been reported regarding this test. The method for reading results is based on visually comparing the amount of fungal growth in the tubes of our microplate with that in the growth-control tubes/wells (without antifungal agent). The MIC can be difficult to interpret for some drugs when using this reading method. For example, amphotericin B exhibits a sharp transition from visible growth to no visible growth at the MIC, and the endpoint is readily apparent and subjective. For the azoles, there can be prominent growth at all drug concentrations, regardless of susceptibility. This may arise because of a delay in the onset of action of these drugs that permits growth before inhibition occurs. The term "trailing" has been used to describe the reduced but persistent growth that some isolates of *Candida* spp. exhibit over an extended range of drug concentrations (Arthington-Skaggs et al. 2002). Hence, it has been suggested that a 50 % rather than an 80 % reduction in growth may provide a better correlation between in vitro data and clinical outcome when using the microdilution method (Rex et al. 1998; St-Germain 2001).

On the other hand, the M27 method is not fully standardized for dimorphic fungi because of the difficulty in culturing them (de Paula e Silva et al. 2013). This occurs with *S. schenckii* complex, *Paracoccidioides* genus, and other dimorphic fungi. In 2002, the M38-A protocol for filamentous fungi was adopted (NCCLS 2002); this is basically a variation of the M27 protocol but it has not yet successfully generated clinically useful MICs. Thus, colorimetric methods such as the microplate alamar Blue assay is proposed as a valuable method to quantitatively measure the proliferation inhibition of dimorphic fungi by antifungal drugs (de Paula e Silva et al. 2013).

The in vitro antifungal susceptibility of different members of *S. schenckii*, and the therapeutic efficacy of several antimycotic drugs has been evaluated in diverse studies. In these studies, *S. schenckii* has shown high variability and relatively high intrinsic antifungal resistance. In general, this variability is attributed to the fact that *Sporothrix* is a complex of different species with genetically diverse strains (Rodrigues et al. 2014). In addition, as sporotrichosis is a worldwide infection, additional variabilities in antifungal susceptibility have been described among isolates from different geographical areas (Silveira et al. 2009). In several reports of in vitro susceptibility, terbinafine was the most active drug compared with itraconazole, ketoconazole, or posaconazole against *S. schenckii sensu strictus* and *S. brasiliensis*, while *S. mexicana* showed the worst response (Kohler et al. 2004; Marimon et al. 2008; Ottonelli et al. 2014). On the other hand, itraconazole has shown good in vitro activity against *S. brasiliensis*, whereas the response was moderate or low for the other species. Rodrigues et al. (2014) demonstrated, in a genetically diverse panel of 68 strains, that the minimum fungicidal concentration of amphotericin B against *S. brasiliensis* and *S. schenckii* was variable, ranging from 2 to >16 μg/mL. This result is in agreement with other reports and would indicate that the susceptibility of this fungus to amphotericin B is dependent on the

strain, and should be studied case by case (Alvarado-Ramírez and Torres-Rodríguez 2007; Silveira et al. 2009). Other assays evaluating flucytosine, caspofungin, and fluconazole found no in vitro antifungal activity against any of the *Sporothrix* species (*S. schenckii*, *S. brasiliensis*, and *S. mexicana*) (Rodrigues et al. 2014).

9.4 Strategies for Human Sporotrichosis Therapy

In light of new knowledge about *Sporothrix* complex species in endemic regions worldwide, and their distinct antifungal sensitivity profiles, differences in treatment might be found. Clinical forms and host immunocompetence must also be considered before treatment initiation.

Itraconazole is the drug of choice for the treatment of cutaneous sporotrichosis (Barros et al. 2004, 2011a, b; Kauffman et al. 2007). This drug is metabolized in the liver, primarily by the cytochrome P450 isoenzyme CYP3A4, so it interacts with other drugs metabolized by this pathway (Katz 1999). More than 200 drug interactions have been reported with the use of itraconazole, which range from mild interactions to absolute contraindications (Micromedex 2014). It is well tolerated, but special care is needed concerning possible interactions and liver damage. The recommended dose for cutaneous sporotrichosis ranges from 100 to 200 mg daily. Capsules are better absorbed if taken after a meal, while the liquid preparation should be taken under fasting conditions (Micromedex 2014). In a study of five patients with cutaneous sporotrichosis treated with itraconazole pulse therapy (400 mg/day for 1 week every month), four patients were cured after an average of three and a half pulses (Bonifaz et al. 2008). In an evaluation of 645 patients, Barros et al. (2011b) demonstrated successful treatment with itraconazole 100 mg daily. In fact, this low dosage is well established for the treatment of patients in Rio de Janeiro, Brazil, where the predominance of *S. brasiliensis* is high (Almeida-Paes et al. 2014).

Terbinafine has been successfully used in the therapy of cutaneous sporotrichosis as an alternative when itraconazole cannot be used (Francesconi et al. 2009); fewer than 20 drug interactions have been reported to date. It is metabolized in the liver and binds some of the cytochrome P450 microsomal enzymes (approximately 5 % of the total capacity), so it is believed not to change the availability of other drugs metabolized by this enzyme system. Terbinafine is renally excreted (80 %) and, in patients with renal and hepatic impairment, its clearance decreases 50 % (Micromedex 2014). Three studies using terbinafine in human patients have demonstrated the efficacy of doses ranging from 250 to 1000 mg daily (Chapman et al. 2004; Francesconi et al. 2009, 2011).

The saturated solution of potassium iodide (SSKI) is still one of the most prescribed drugs for the treatment of cutaneous sporotrichosis, due to its effectiveness and low cost. Its mechanism of action is unknown, but it has been suggested that this salt acts in the resolution of the granulomas by increasing the proteolysis.

Other researchers suggest that it promotes increased phagocytosis (Barros et al. 2011a; Reis et al. 2012b). In vitro studies suggest that it promotes the destruction of the yeast wall during the conversion from iodide to iodine (Torres-Mendoza et al. 1997). SSKI is initially administered at a dosage of 5 drops three times daily and gradually increased, as tolerated, to 40–50 drops three times daily. The dosage for children is 1 drop three times daily, increased as tolerated up to 1 drop/kg (maximum of 40–50 drops) three times daily (Kauffman et al. 2007). It can be taken with water or, preferably, juice or milk. Frequently reported adverse effects include metallic taste, nausea, vomiting, anorexia, epigastric pain, and diarrhea. These effects can be attenuated by dose reduction or temporary discontinuation of the drug. With prolonged use, some patients may experience symptoms of iodism (pronounced metallic taste, burning in the mouth, sialorrhea, teeth and gum sensitivity, and headache) or potassium toxicity (arrhythmias, weakness, mental confusion, and paresthesia) (Sterling and Heymann 2000).

Sensitivity studies demonstrated good in vitro activity of itraconazole, terbinafine, and posaconazole against *Sporothrix* spp. (Meinerz et al. 2007; Gutierrez-Galhardo et al. 2008; Silveira et al. 2009). Posaconazole is a second-generation triazole with a broad spectrum, but its use in sporotrichosis remains to be assessed (Scheinfeld 2007; Silveira et al. 2009). In a study of mice, this drug was able to prevent death by *S. brasiliensis* and *S. schenckii*, and reduce the tissue fungal burden to levels close to those achieved with amphotericin B (Fernandez-Silva et al. 2012). Recently, posaconazole has been successfully used in combination with amphotericin B in the treatment of disseminated sporotrichosis by *S. schenckii* in a patient with hairy cell leukemia (Bunce et al. 2012). Its recommended dosage for sporotrichosis is not established yet, but for other invasive and disseminated mycoses it varies from 200 mg three times daily to 400 mg twice daily, depending on the clinical form of the patient and on the response to previous treatment with other azoles (Merck 2014).

The treatments of choice for the severe disseminated forms of sporotrichosis are itraconazole at higher dosages (200 and 400 mg daily) or amphotericin B. The latter is effective in intravenous infusion at a dosage of 0.25–1 mg/kg/day in cumulative dosages of 2–4 g according to clinical response. However, numerous adverse effects can be observed, including renal and cardiac toxicity. The colloidal dispersion or liposomal presentations of the drug induce adverse reactions similar to those with the presentation of conventional deoxycholate, but are less frequent and less severe, which allows the use of higher doses, therefore shortening the treatment time (Kauffman et al. 2007).

9.4.1 Adjuvant Treatment

Adjuvant treatments can be successfully employed in various situations. Local application of heat (42–43 °C) from hot water, infrared light, or similar methods for approximately 15–20 min three times daily is indicated in nodular and fistulized lesions. This form of treatment is based on the sensitivity of the fungus to high temperatures and is an important therapeutic alternative in pregnant women and in

those at increased risk with the use of drugs (Rippon 1988; Kwon-Chung and Bennett 1992; Barros et al. 2001, 2004; Lacaz 2002; Rosa et al. 2005; Kauffman et al. 2007). Solutions containing iodine are used in ulcerated lesions, with regard for the sensitization to this element and the extent of the injuries. Massive iodine absorption has deleterious effects in patients with renal insufficiency.

In sporotrichosis, the persistence of infiltrated and residual lesions despite healing of other lesions is common. Curettage and/or electrocoagulation of these lesions often allows for the suspension of antifungals (Valle and Gutierrez-Galhardo 2012). Another method with excellent results is cryotherapy of the verrucous or ulcero-vegetative isolated lesions with liquid nitrogen (Ferreira et al. 2011). Cryotherapy can also be employed in multiple skin lesions associated with AIDS. This method partially destroys the lesion and improves drug delivery. The aspiration of fluctuant lesions relieves pain and promotes healing (Valle and Gutierrez-Galhardo 2012). Recently, photodynamic therapy adjunctive to itraconazole was successfully employed to treat a localized verrucous lesion of sporotrichosis resistant to treatment with itraconazole and terbinafine (Gilaberte et al. 2014).

9.4.2 Treatment During Pregnancy

Pregnant women, if possible, should not receive drug treatment until the end of pregnancy. In lymphocutaneous and fixed cutaneous forms, local heat can be applied successfully. If the lesions persist, pharmacological treatment can then be initiated after cessation of breast-feeding, as the drugs pass into breast milk and may cause harm to the infant. Itraconazole is contraindicated during pregnancy and breastfeeding due to its potential teratogenicity; terbinafine has no major contraindications but should also be avoided (Kauffman et al. 2007). In case of disseminated or severe disease, amphotericin B should be employed (Valle and Gutierrez-Galhardo 2012).

9.4.3 Treatment of HIV/AIDS Patients

Sporotrichosis patients co-infected with HIV should be treated according to their clinical forms and to their immune status. Immunocompetent patients evaluated as having a T $CD4^+$ cell count $\geq$200/μL tend to present with localized sporotrichosis and can receive regular doses of itraconazole or terbinafine. In contrast, immunosuppressed patients (T $CD4^+$ cell count $<$200/μL) frequently develop disseminated sporotrichosis and must receive intravenous amphotericin B and/or oral itraconazole 400 mg daily, as recommended for all patients with severe and disseminated sporotrichosis. The difference in the treatment of HIV/AIDS patients is in the extension of a suppressive dosage of 200 mg daily of itraconazole after the patient is considered cured, until the $CD4^+$ cell count is consistently above 200/μL (Kauffman et al. 2007; Freitas et al. 2012, 2014) (Table 9.1).

Table 9.1 Summary of guidelines for the treatment of various forms of human sporotrichosis (Kauffman et al. 2007)

Clinical manifestations	Preferred treatment [dose]	Alternative treatment	Remarks
Uncomplicated cutaneous sporotrichosis	Itraconazole [200 mg/day]	Itraconazole [200 mg b.i.d.] or terbinafine [500 mg b.i.d.], or SSKI [increasing doses], or fluconazole [400–800 mg/day] or local hyperthermia	Treat for 2–4 weeks after lesions have resolved
Osteoarticular sporotrichosis	Itraconazole [200 mg twice daily (bid)]	Liposomal amphotericin B (3–5 mg/kg/day) or deoxycholate Amphotericin B [0.7–1 mg/kg/day] until resolution	Switch to itraconazole after resolution and treat for a total of 12 months
Pulmonary sporotrichosis	Liposomal amphotericin B [3–5 mg/kg/day] then itraconazole [200 mg bid]	Deoxycholate amphotericin B [0.7–1 mg/kg/day] until recovery then, Itraconazole [200 mg b.i.d.]	Treat less severe disease with itraconazole. Treat for at least 12 months
Meningeal sporotrichosis	Liposomal amphotericin B [3–5 mg/kg/day] then itraconazole [200 mg bid]	Deoxycholate amphotericin B [0.7–1 mg/kg/day] until recovery, then Itraconazole [200 mg b.i.d.]	Length of therapy with amphotericin B is not established. Treat for 4–6 weeks and total of 12 months. Suppressive therapy with itraconazole needed
Disseminated sporotrichosis	Liposomal amphotericin B [3–5 mg/kg/day], then itraconazole [200 mg bid]	Deoxycholate amphotericin B [0.7–1 mg/kg/day] until recovery, then itraconazole [200 mg b.i.d.]	Treat with amphotericin B until objective improvement, and for at least 12 months. Suppressive therapy with itraconazole needed
Sporotrichosis in pregnant women	Treat only severe sporotrichosis with liposomal amphotericin B [3–5 mg/kg/day] or deoxycholate amphotericin B [0.7–1 mg/kg/day]. Treat with local hyperthermia [approx 45 °C] for uncomplicated cutaneous sporotrichosis.		Preferably, defer treatment for uncomplicated cases
Sporotrichosis in children	Itraconazole [6–10 mg/kg/day or maximum 400 mg/day] for mild disease, deoxycholate amphotericin B [0.7–1 mg/kg/day] for severe disease	SSKI with increasing doses equivalent to half the adult dose for a duration as in adults	Treat severe disease with an amphotericin B formulation

bid twice per day; *SSKI* saturated solution potassium iodide

9.5 Treatment of Sporotrichosis in Cats and Dogs

Treatment of dogs and cats with sporotrichosis still represents a challenge for veterinarians, as oral antifungal agents are scarce and may have adverse effects (Pereira et al. 2014). The cost and availability of antifungal treatments vary and often dictate the choices made (Crothers et al. 2009).

In the management of sporotrichosis in animals, clinical and biochemical monitoring is strongly recommended (Rosser and Dunstan 2006), regardless of the prescribed treatment regimen. The use of immunosuppressive drugs such as glucocorticoids is contraindicated both during and after the treatment of the disease. Any concurrent bacterial infection should be simultaneously treated (Rosser and Dunstan 2006).

9.5.1 Feline Sporotrichosis

Iodides, ketoconazole, itraconazole, amphotericin B, local heat therapy, and surgical removal of the lesions are the current treatment options available for cats with sporotrichosis (Gremião et al. 2015). The azoles (itraconazole and ketoconazole) and potassium iodide (KI) are the most common drugs for the treatment of feline sporotrichosis (Pereira et al. 2010; Reis et al. 2012a). Clinical cure is observed regardless of the initial clinical findings or co-infection with feline immunodeficiency virus (FIV) and/or feline leukemia virus (FeLV) (Fig. 9.3). Treatment may take a few weeks to several months (median time 4–9 months) and must be continued for at least 1 month after clinical cure (Pereira et al. 2010). Remission of the lesions without antifungal treatment is rare (Schubach et al. 2004).

Exogenous re-infection and recurrence after clinical cure may occur. The latter demonstrates the possibility of reactivation of the lesions even when the

Fig. 9.3 Feline sporotrichosis. (**a**) Ulcerative lesions and crusts on the nasal bridge and left ear with drainage of sera sanguinolent exudate; (**b**) Scar tissue on the bridge of the nose after azole antifungal treatment

recommended treatment has been completed (Chaves et al. 2013). Fatal outcomes have been reported (Schubach et al. 2004; Pereira et al. 2010; Reis et al. 2012a).

Traditionally, KI and sodium iodide (NaI) (10–20 mg/kg every 12–24 h) have been used in the treatment of sporotrichosis. Nonetheless, serious adverse effects associated with the use of iodides in cats, such as lethargy, anorexia, vomiting, diarrhea, spasms, hypothermia, and cardiomyopathy, have led to their replacement with safer and more effective antifungal drugs, such as the azoles. In feline sporotrichosis, reports of cases treated with NaI and SSKI are few and the results inconclusive (Pereira et al. 2009).

Adverse effects with the use of iodides in cats, although common, do not preclude its application, as they are reversible with drug suspension or dose decrease. Thus, a study evaluated the effectiveness of KI in cats with sporotrichosis. Potassium iodide was manipulated in capsules, as it is convenient and easy to administer compared with SSKI. The dose used varied from 2.5 to 20 mg/kg every 24 h. The scaling period of the drug was initially established with 2.5 mg/kg every 24 h. Doses were then progressively increased at each 5-day period until a clinical response was achieved or signs of toxicity appeared, as follows: 5, 10, 15, and 20 mg/kg every 24 h. The KI cure rate was 47.9 %, and the clinical adverse effects, principally hyporexia, were observed in 52.1 % of cases. Continuous KI therapy could lead to interruption of the endogenous production of thyroid hormones and may also cause thyroiditis and/or hypo- or hyperthyroidism. Thus, thyroid-stimulating hormone screening is prudent to ensure that thyroid function remains normal after 1 month of treatment. Based on the positive responses seen in cats treated with KI capsules and on the low cost of this drug, it should be considered as another treatment option (Reis et al. 2012a). Itraconazole as monotherapy or in combination with KI is an alternative for cats that do not respond to KI therapy.

Ketoconazole may be used for the treatment of feline sporotrichosis (5–27 mg/kg every 24 h). This drug has poor selective toxicity, that is, it inhibits cytochrome P450 both in the fungus and in mammals, resulting in side effects, the most common being anorexia, depression, nausea, vomiting, diarrhea, and elevated liver enzymes (Heit and Riviere 1995; Rosser and Dunstan 2006).

The effectiveness and safety of treatment with ketoconazole 50–100 mg/cat every 12 or 24 h (13.5–27 mg/kg every 12 or 24 h) were evaluated in sporotrichosis-infected cats. Clinical cure was achieved in 171 (28.6 %) of the 598 cats, with a median treatment time of 28 weeks. Of the 598 cats treated, 252 (42.1 %) presented with gastrointestinal adverse effects, hyporexia being the most frequent, followed by vomiting and/or diarrhea (Pereira et al. 2010). Currently, ketoconazole has been replaced by itraconazole in cats.

Itraconazole is considered the drug of choice in the treatment of feline sporotrichosis (Gremião et al. 2015). The oral liquid preparation is preferred to the capsules because it permits more accurate dose measurement and improved absorption and bioavailability at the recommended dose. Therefore, the dosages of oral solution per unit of body weight are lower than for itraconazole capsules. Capsules can be opened, and a fraction of the dose can be mixed in food; however, beads should

remain intact (Greene and Calpin 2012). The adverse effects observed in cats treated with this drug are similar to those described for ketoconazole (Pereira et al. 2010).

In a study that included 175 cats treated with itraconazole 50–100 mg/cat every 12 or 24 h (8.3–27.7 mg/kg every 24 h), 67 cats (38.3 %) achieved complete resolution of clinical signs, with a median treatment time of 26 weeks. Higher doses were used because of the difficulty in achieving clinical cure with doses recommended in the literature (5–10 mg/kg every 24 h). Gastrointestinal adverse effects were observed in 54 cats (30.9 %), mainly hyporexia. Itraconazole was more effective than ketoconazole, and was associated with fewer gastrointestinal adverse when the effectiveness and safety of these drugs were compared in 773 cats with sporotrichosis (Pereira et al. 2010).

Cases of cats with refractory sporotrichosis despite treatment with conventional oral antifungals have been described. In those cases, KI capsules as monotherapy or combined with itraconazole as well as intralipid or subcutaneous amphotericin B might be an alternative (Gremião et al. 2015).

Of 14 cats with sporotrichosis presenting persistent lesions refractory to itraconazole or ketoconazole, 12 achieved clinical cure after treatment exclusively with KI capsules (2.5–20 mg/kg every 24 h) (Reis et al. 2012b). Of 31 cats with sporotrichosis presenting lesions refractory to treatment with oral itraconazole (100 mg/day), 24 achieved clinical cure with a combination of KI capsules (5 mg/kg/every 24 h) and itraconazole (100 mg/every 24 h) (Rocha 2014).

Reports on the administration of amphotericin B for the treatment of feline sporotrichosis are scarce. Intravenous administration of the drug in cats is limited because of serious adverse effects, and no descriptions are available of clinical cure in cats with sporotrichosis. Intralipid administration of amphotericin B, rather than intravenous, combined with oral itraconazole in a cat with a skin lesion in the nasal region refractory to a triazole was successfully used without adverse effects (Gremião et al. 2009). The same therapeutic protocol was used in 26 cats with residual localized skin lesions refractory to itraconazole. Clinical cure was achieved in 72.7 % of the cats and 27.3 % experienced recurrence of the lesions at the same site (Gremião et al. 2011).

Therapeutic response to subcutaneous amphotericin B combined with oral itraconazole was described in 17 cats with sporotrichosis presenting skin and/or mucosal lesions refractory to oral itraconazole. Treatment was effective in 35.3 % of cases; however, relapse, lack of clinical response, and worsening of lesions also occurred, as well as formation of local sterile abscesses (Gremião et al. 2015).

Lipid formulations of amphotericin B exhibit less nephrotoxicity than the conventional drug, and their use is indicated for the treatment of disseminated forms of sporotrichosis. Successful use of intravenous liposomal amphotericin B combined with oral itraconazole was described in a case of feline sporotrichosis refractory to oral itraconazole. However, the cost of these compounds remains high and may represent a limiting factor to their use (Gremião et al. 2015).

Terbinafine shows good performance in vitro against isolates of *Sporothrix* sp. and the therapeutic potential has been confirmed in humans (Francesconi et al. 2009). Fluconazole is considered only moderately effective in human sporotrichosis (Kauffman et al. 2007). Posaconazole has shown good performance in antifungal susceptibility tests, and may represent an alternative for the treatment of systemic or severe sporotrichosis (Marimon et al. 2008). To the best of our knowledge, the effectiveness of these drugs has not been studied in the treatment of feline sporotrichosis.

Other options for the treatment of feline sporotrichosis include thermotherapy, adjunctive surgical therapy, and cryosurgery. Local hyperthermia was successfully described in a cat presenting a single ulcer in the thoracic region (Honse et al. 2010). The surgical resection of lesions combined with antifungal therapy can be an alternative after medical treatment has failed in cats with sporotrichosis (Gremião et al. 2006; Hirano et al. 2006). Cryotherapy has been encouraged as a complementary therapy in feline sporotrichosis (Malik et al. 2009; Gremião et al. 2011). The use of this therapy in combination with oral itraconazole was effective in a cat with sporotrichosis that presented a persistent refractory skin lesion (Gremião et al. 2015).

In Brazil, authors have described difficulties in handling cats with sporotrichosis, including administering oral medication to cats, keeping the animals confined, and using public transport to take the animals to clinic, as major causes of treatment abandonment (Barros et al. 2010). Cessation of treatment mainly occurs when the cat owner observes the healing of skin lesions. In addition, the acquisition of the disease by a family member and the requirement for long-term treatment are factors that can lead owners to cease treatment and request euthanasia of the cat (Chaves et al. 2013).

The prognosis for cats depends on the number, extension, and location of the lesions, the occurrence of respiratory signs, and the general medical condition. Feline sporotrichosis is hard to treat and requires a long period of daily care, and cats do not always respond well to treatment. The cooperation and persistence of the owners is necessary for successful treatment (Gremião et al. 2015).

9.5.2 Canine Sporotrichosis

Generally, canine sporotrichosis has a good prognosis compared with feline disease. Treatment may take a few weeks to several months, and must be continued for at least 1 month after clinical cure (Schubach et al. 2012). Schubach et al. (2006) described remission of lesions without antifungal treatment in five dogs. In the past, the treatment of choice in dogs with sporotrichosis was the oral administration of SSKI (Rosser and Dunstan 2006); however, ketoconazole and itraconazole have replaced iodide therapy.

Ketoconazole is usually well tolerated, and itraconazole has been used in animals that cannot tolerate or that fail to respond to iodides and ketoconazole (Rosser and Dunstan 2006). Acid environment, fats, and smaller amounts of food generally enhance absorption of azoles. Some authors described the clinical cure of dogs using ketoconazole (5–10 mg/kg/every 12–24 h) and itraconazole (5–10 mg/kg/every 12–24 h) (Rosser and Dunstan 2006; Schubach et al. 2006; Whittemore and Webb 2007; Madrid et al. 2007; Rossi et al. 2013). The duration of treatment ranged from 2 to 15 months (median 3.5 months) with ketoconazole, and 2–5 months (median 2.5 months) with itraconazole (Schubach et al. 2006).

The most common adverse effects of the azoles are hyporexia, anorexia, vomiting, diarrhea, and an increase of hepatic enzyme levels. The potential hepatotoxicity of ketoconazole and itraconazole should be monitored during therapy. Moreover, ketoconazole may reduce the production of cortisol and testosterone in dogs at doses greater than 10–30 mg/kg every 24 h (Greene and Calpin 2012), and the development of vasculitis as a result of itraconazole therapy has been described (Rosser and Dunstan 2006; Schubach et al. 2006).

9.6 Current and Future Approaches to Improving the Management of Sporotrichosis

The lengthy period of treatment often associated with fungal resistance and toxicity has generated a growing interest in developing methods to improve the efficacy and safety of therapy. Some strategies are being evaluated, even more are in experimental phases: (1) use of vehicles for classic antifungals; (2) combination with immunostimulants; (3) vaccine development, (4) use of non-conventional antifungals.

9.6.1 Use of Vehicles for Classic Antifungals

As many antifungals have historically been hampered by toxicity, availability, and solubility issues, several formulations have been developed and are currently in clinical use or under preclinical investigation to overcome these problems. Different lipid-containing formulations of amphotericin B have been developed in an attempt to attenuate its nephrotoxicity and increase its therapeutic potential. For the past decades, investigators have evaluated the use of phospholipid vesicles known as liposomes as a target drug. Lopez-Berestein et al. (1984) developed the first liposomal formulation of amphotericin B trialled in humans. In 1995, amphotericin B lipid complex, or ABLC (Abelcet; The Liposome Co., Princeton, NJ, USA), became the first lipid-formulated amphotericin B product to receive approval from

the US Food and Drug Administration (FDA). Subsequently, amphotericin B colloidal dispersion (ABCD; Amphotec; Sequus Pharmaceuticals, Menlo Park, CA, USA), another lipid-formulated amphotericin B product dispersed in water received FDA approval in 1996. A third product, AmBisome (L-AmB; NeXstar Pharmaceuticals/Fujisawa, San Dimas, CA, USA), long commercially available outside of the USA, received FDA approval in 1997 (Wong-Beringer et al. 1998).

Liposomal amphotericin B is a lipid-associated formulation of amphotericin B and is approved for the treatment of invasive fungal infections in many countries worldwide (Moen et al. 2009), and accepted for use in sporotrichosis (Kauffman et al. 2007). This formulation was developed to improve the tolerability profile of amphotericin B deoxycholate, which was for many decades considered the gold standard of antifungal treatment despite being associated with infusion-related events and nephrotoxicity. For the treatment of confirmed invasive fungal infections, liposomal amphotericin B was more effective than amphotericin B deoxycholate treatment in patients with disseminated sporotrichosis and AIDS. Despite being associated with fewer infusion-related adverse events and less nephrotoxicity than amphotericin B deoxycholate and amphotericin B lipid complex, liposomal amphotericin B use is still limited to some extent by these adverse events. The cost of liposomal amphotericin B therapy may also restrict its use, but further pharmacoeconomic studies are required to fully define its cost effectiveness in comparison with other antifungal agents. Liposomal amphotericin B also shows immunomodulatory effects, although the mechanisms involved are not fully understood and differ from those of amphotericin B deoxycholate. In adult patients with febrile neutropenia, the pharmacokinetics of intravenous liposomal amphotericin B are nonlinear, with higher than dose-proportional increases in exposure being consistent with reticuloendothelial saturation and redistribution of amphotericin B in the plasma compartment. Liposomal amphotericin B is rapidly and extensively distributed after single and multiple doses, with steady-state concentrations of amphotericin B attained within 4 days and no clinically relevant accumulation of the drug following multiple doses of 1–7.5 mg/kg/day. In autopsy tissue, the highest concentrations of the drug were found in the liver and spleen, followed by the kidney, lung, myocardium, and brain tissue.

Cochleates are a lipid-based delivery vehicle consisting of crystalline phospholipid-cation structures that form spiral lipid sheets. These unique structures were first described by Papahadjopoulos and Wilschut (1979) and later by others (Goldstein and Lukaynov 1997; Lee and Lukaynov 1998; Lee and Carlson 1999; Price and Patchan 1991). They represent a new technology platform for oral delivery of clinically important drugs that possess poor oral bioavailability. Utilization of cochleates to deliver drugs such as anti-fungal agents, DNA, and subunit vaccines were reported by Mannino et al. (1998) and Zarif and Mannino (2000). Orally administered cochleates containing amphotericin B (CAMB) showed broad-spectrum activity in murine infection models of candidiasis (Zarif et al. 2000; Santangelo et al. 2000), aspergillosis (Delmas et al. 2002), and cryptococcosis

(Perlin 2004). Initial biodistribution studies of CAMB administered orally in mice demonstrated that cochleates delivered significant levels of amphotericin B to target organs. The lipid particulate nature of cochleates also imparted reduced toxicity that mimics other lipid amphotericin B complexes. Cochleates are a promising new vehicle for oral delivery of amphotericin B at therapeutic levels (Perlin 2004; Rao et al. 2007; Syed et al. 2008).

Nanoparticle formulation technology has revolutionized drug delivery and release, and are being evaluated as a carrier of antifungals (Constantinides et al. 2008; Van de Ven et al. 2012). These formulations include various nanoparticles of amphotericin B has and have shown good in vitro and in vivo activity with decreased toxicity, even by lipid formulation standards and are potential candidates for oral administration (Jung et al. 2009; Italia et al. 2009; Patel and Patravale 2011). To date, no reports are available of nanoformulations evaluated against *S. schenckii* complex.

9.6.2 Combination with Immunostimulants

The advances in understanding of fungal pathogenesis and the induction of protective antifungal adaptive immunity have enabled the development of immunotherapeutic strategies and vaccines against invasive and localized mycosis (Kullberg et al. 2014). Thus, a valuable approach to improve antifungal therapy is combination with immunostimulants (immunotherapy). The use of cytokines and diverse immunomodulators as therapeutic tools has been evaluated against different pathogenic fungi with success (Stevens 1998; Steinbach and Stevens 2003; de Sousa et al. 2014).

A recent study investigated whether treatment with recombinant murine IL-12 (rmIL-12) promoted type 1 T helper (Th1) immunity and/or clinical improvement in an experimental sporotrichosis gerbil model. Gerbils were inoculated with *S. schenckii* in the footpad and treated with rmIL-12. A significant increase in macrophage phagocytosis and oxidative burst, and in delayed-type hypersensitivity (DTH) reaction in rmIL-12 treated gerbils 7 days post-infection, as well as a significant increase of serum interferon (IFN)-γ and a decrease of IL-4 and IL-10. Moreover, rmIL-12 substantially decreased *S. schenckii* burden in liver and spleen and improved the clinical outcome preventing footpad ulcer and tail nodules observed in untreated gerbils (Flores-García et al. 2015). Moreover, Guterres et al. reported the use of (1–3) β-glucan along with itraconazole in the treatment of ulcerated and crusted lesions, especially on the nasal planum in a dog with sporotrichosis caused by *S. brasiliensis*. After 7 months of treatment with itraconazole, the *S. brasiliensis* culture was still positive, so itraconazole was combined with (1–3) β-glucan. After a weekly application for 4 weeks of glucan (subcutaneous application), complete elimination of the fungus was observed

based on a negative fungal culture result (Guterres et al. 2014). According to these experiences, immunotherapy might be a promising therapeutic alternative in cases of resistance to conventional therapy. More studies are necessary to evaluate different immunotherapy modalities in controlled assessments of sporotrichosis.

9.6.3 Vaccine Development

The use of vaccines to neutralize fungal virulence factors is an exciting new strategy that is only in its infancy. The concept of an antifungal vaccine has been researched for over 30 years. Numerous antifungal experimental vaccines are in different developmental phases, but thus far no fungal vaccine is available for clinical use (Edwards 2012; Portuondo et al. 2015a; Nanjappa and Klein; 2014). As with other diseases caused by eukaryotic organisms such as malaria, development of an effective fungal vaccine is difficult because the organisms are more complex than bacterial or viral pathogens. A second challenge specific to fungi is the lack of understanding of natural fungal immunity and the relative contributions of the innate acquired cellular and acquired humoral arms. Further challenges include an under-appreciation of the magnitude of fungal infections from both opportunistic and endemic fungi, as well as other logistical reasons such as inadequate staffing of offices for technical transfer in many universities, the competitiveness of small business innovative research and small business technical transfer grants, the high cost of preparing antigens for use in human studies to meet standards for good manufacturing process, the high cost of toxicology studies and of conducting phase I clinical trials to establish tolerability and safety (Edwards 2012).

In spite of these difficulties, research in antifungal vaccines continues. Some attempts have been made to obtain an anti-sporothrix vaccine. de Souza et al. (2011) reported the effects of gamma radiation on the yeast of *S. schenckii* with a view to developing a radioattenuated vaccine for veterinary use, but no other advances in this research have been published thus far. More recently, several anti-sporothrix vaccine candidates have been developed in Carlos's laboratory (UNESP). In this way, the immunogenicity and protective properties of different adjuvanted *S. schenckii* cell wall protein (ssCWP)-based vaccine formulations in a mouse model of systemic *S. schenckii* infection was assessed. Sera from immunized mice recognized different ssCWP. Furthermore, opsonization with the anti-ssCWP sera led to markedly increased phagocytosis and was able to strongly inhibit the fungus' adhesion to fibroblasts. Immunization with the adjuvanted formulations led to increased ex vivo release of IL-12, IFN-γ, IL-4, and IL-17, whereas only IL-12 and IFN-γ were induced by the higher-dose non-adjuvanted formulation. Lastly, passive transference of the anti-ssCWP serum from vaccinated mice was able to afford in vivo protection in a subsequent challenge with *S. schenckii*, becoming a

viable vaccine candidate for further testing (Portuondo 2015b). More studies on these vaccine candidates and others based on activated dendritic cells are ongoing in our laboratory.

Keeping in mind that sporotrichosis is an opportunistic mycosis, a possible limitation to the use of vaccines in immunosuppressed patients is that these patients may not develop protective responses. On the other hand, passive immunization with protective antibodies may well be a rapid and effective preventive or even therapeutic alternative (Almeida 2012). Encouraging results were shown by de Almeida's group in two studies using a monoclonal antibody (mAb) against a 70-kDa glycoprotein (gp70) from *S. schenckii* as a therapeutic vaccine in mice infected with highly virulent *S. schenckii* and *S. brasiliensis*, resulting in the reduction of fungal burden in spleen and liver (Nascimento et al. 2008; de Almeida et al. 2015). As gp70 has an adhesin function, the authors explained that this mAb could inhibit *S. schenckii* adhesion to host tissues and/or the extracellular matrix and consequently abort infection. In addition, passive transference could increase the cell-mediated immune response and IFN-γ production (Nascimento et al. 2008). Thus, they showed that yeast cells opsonized with mAbs against gp70 exhibited an increased phagocytic index and TNF-α production (Franco et al. 2012).

Other experiences using mAbs as alternative antifungal management have been explored, demonstrating protective activities against several fungi in laboratory studies (Mukherjee et al. 1994; Goldman et al. 1997; Xander et al. 2007; Buissa-Filho et al. 2008; Toledo et al. 2010; Zhang et al. 2011). Other valuable results have been obtained in human mycosis using specific therapeutic mAbs, mainly against cryptococcosis (Larsen et al. 2005) and candidiasis (Pachl et al. 2006; Krenova et al. 2010). The efficacy of therapeutic mAbs would be augmented when they are used in combination with conventional antifungal therapy.

More studies exploring different choices of immunoprophylaxis and immunotherapy associated with other established schedules are necessary. It is an exciting challenge that may lead to novel approaches in the management of sporotrichosis.

9.6.4 Use of Non-Conventional Antifungals

Photodynamic therapy involves the use of harmless visible light combined with a photosensitizer in the presence of oxygen, which generates reactive oxygen species, with a cytotoxic effect against microorganisms. This therapy has been proposed as an alternative approach for localized infections, including localized cutaneous and subcutaneous mycosis (Dai et al. 2012). This method, used as an adjuvant treatment in parallel with conventional antifungal therapy, can improve the efficacy of the chemotherapy, with a faster infection cure and lower toxicity (Qiao et al. 2010; Lyon et al. 2011). Gilaberte et al. (2014) reported a case of cutaneous sporotrichosis treated with different antifungal treatments with partial improvement accompanied

with toxicity signs. The patient was successfully treated with photodynamic therapy, and in vitro photosensitivity of the *S. schenckii* yeast isolated from the patient was confirmed. This report encourages the development of new research to evaluate the usefulness of this alternative therapy for sporotrichosis.

In recent years, the antimicrobial properties of medicinal plants have been increasingly reported in different parts of the world. Factors contributing to the growing interest in searching for medicinal plants with antifungal effects include the economic crisis, high costs for industrialized medicines, inefficient public access to medical and pharmaceutical care, and the side effects caused by synthetic drugs (Johann et al. 2007).

Plants constitute an invaluable source of antifungal compounds because of their unmatched chemical diversity, and much effort worldwide has been put into identifying medicinal plants with antifungal properties (Rai and Mares 2003; Daboit et al. 2010; Maregesi et al. 2008), some of them in Latin America (Agüero et al. 2007; Cruz et al. 2007; Danelutte et al. 2003; Escalante et al. 2002; Malheiros et al. 2005; Svetaz et al. 2010), Asia (Kalaskar et al. 2012; Zhang et al. 2013; Duan et al. 2013; Wen et al. 2014), and Africa (Mokoka et al. 2010; Zarai et al. 2011; Otang et al. 2012; Adamu et al. 2012; Bouzabata et al. 2013).

Diverse types of plants have been evaluated against *S. schenckii* and other fungi. Some of these reports are summarized in Table 9.2.

9.7 Prevention

The main objective in preventing sporotrichosis is avoiding mold spores from entering the skin. Gardeners, farmers, agriculturists, and other individuals in contact with roses, hay, sphagnum moss, or soils, should use heavy boots and gloves to prevent puncture wounds and should cover any scratches or wounds in their skin. These measures also apply to builders, carpenters, and others working in the outdoors with risks for skin wounds. People with immunological deficiency should be exceptionally careful to avoid any contact with rose thorns or soil and moss used for gardening or on farms.

As a preventive measure, cats should be kept indoors in endemic areas to avoid contact with infected or carrier cats; this is especially important for immunocompromised animals (e.g., retrovirus-infected cats or those receiving immunosuppressive drugs). Pet owners moving to endemic areas such as Central and South America should be warned about sporotrichosis, especially if cats are to be allowed to have access to the outdoors. Veterinarians dealing with infected or suspicious cats must use preventive measures to avoid infection, taking extreme care with hygiene, using gloves and sanitary clothes and avoiding direct contact with sources of infection as well as frequently disinfecting the workplace (Lloret et al. 2013).

Table 9.2 Studies of antifungal effect of plants formulation against *Sporothrix schenckii*

Plants	Type of formulations	Fungi evaluated	Main antifungal results against *S. schenckii*	References
Schinus terebinthifolius	Ethanolic extract from the leaves	*Candida albicans ATCC 18804*, *C. krusei ATCC 20298*, *C. tropicalis ATCC 750*, *C. parapsilosis ATCC 22019*, *C. glabrata ATCC 2001*, *Sporothrix schenckii ATCC 20679* and *Cryptococcus neoformans ATCC 32608*.	MIC: 15 μg/mL. The higher in vitro antifungal activity compared with the other evaluated fungi	Johann et al. (2007)
Piper abutiloides Kunth.	Hydroalcoholic extract of aerial parts	*C. albicans*, *C. parapsilosis*, *C. krusei*, *C. glabrata*, *C. tropicalis*, *C. neoformans* and *S. schenckii*	MIC: 125 μg/mL	Johann et al. (2009)
Curcuma longa L.	Turmeric oil	*S. schenckii; Fonsecaea pedrosoi* (clinical isolates)	MIC: 114.9 μg/mL	Apisariyakul et al. (1995)
Pterocaulon polystachyum	Crude methanolic of aerial parts	*24 S. schenckii* (clinical isolates)	MIC range of 156 and 312 μg/mL	Stopiglia et al. (2011)
Bourreria huanita (Lex.) Hemsl	Ethanolic extract of flowers	*S. schenckii; F. pedrosoi* (clinical isolates)	MIC: 12.5 μg/mL	Gaitán et al. (2011)
Phytolacca bogotensis Kunth	Dichloromethanolic extract of leaves		MIC: 12.5 μg/mL	
Gnaphalium gaudichaudianum DC	Methanolic extracts of aerial parts		MIC: 50 μg/mL	
Piper scabrum Lam	Ethanolic extract of leaves		MIC: 25 μg/mL	
Monnina xalapensis Kunth	Ethanolic extract of leaves or stalk		MIC: 50 μg/mL (leaves) MIC: 12.5 μg/mL (stalk)	
Crataegus pubescens (C. Presl.) C. Presl.	Ethanolic extract of fruits		MIC: 12.5 μg/mL	

(continued)

Table 9.2 (continued)

Plants	Type of formulations	Fungi evaluated	Main antifungal results against *S. schenckii*	References
Amyris pinnata Kunth	Ethanolic extract of leaves		MIC: 50 μg/mL	
Cestrum parqui L'Her	Methanolic of fruits		MIC: 50 μg/mL	
Terminalia. triflora	Ethanolic extract of leaves		MIC: 25 μg/mL	
Lippia graveolens Kunth	Ethanolic extract of leaves		MIC: 25 μg/mL	
Aristolochia gibertii Hook.	Methanolic extracts of leaves and flowers		MIC: 50 μg/mL	
Polymnia maculata Cav	Ethanolic extract of leaves		MIC: 50 μg/mL	
Zuccagnia punctata Cav.	Methanolic extracts of leaves		MIC: 50 μg/mL	
Agapanthus africanus extractives	Ethanolic extract of the rhizomes	*Trychophyton mentagrophytes* and *S. schenckii*	MIC: 15.6 μg/mL	Singh et al. (2008)
Cestrum auriculatum	Ethanolic extract of leaves	*S. schenckii IMTAvH 36836* (clinical isolates)	Growth inhibition zone diameter: 25 mm	Rojas et al. (2003)
Croton ruizianus Iryanthera lancifolia Wigandia urens	Ethanolic extract of leaves or stems		Growth inhibition zone diameter: 13 mm	
Oenothera multicaulis	Ethanolic extract of aerial parts, root		Growth inhibition zone diameter: 19 mm (roots); 15 mm (aerial parts),	
Ophryosporus peruvianus	Ethanolic extract of aerial parts, root		Growth inhibition zone diameter: 19 mm	
Senecio culcitioides	Ethanolic extract of aerial parts		Growth inhibition zone diameter: 15 mm	

Terminalia prunioides *Terminalia brachystemma* *Terminalia sericea* *Terminalia gazensis* *Terminalia mollis* *Terminalia sambesiaca*	Leaves extracts using: acetone, hexane, dichloromethane or methanol (separately)	*S. schenckii* clinical isolate from a horse with cutaneous lymphangitis)	MIC: 0.32 (A), 0.32 (H), 0.64 (D), 0.32 (M) mg/mL MIC: 0.32 (A), 0.64 (H), 0.32 (D), 0.64 (M) mg/mL MIC: 0.32 (A), 0.64 (H), 0.64 (D), 0.32 (M) mg/mL MIC: 0.08 (A), 0.16 (H), 0.16 (D), 0. 16 (M) mg/mL MIC: 0.08 (A), 0.16 (H), 0.04 (D), 0.02 (M) mg/mL MIC: 0.04 (A), 0.04 (H), 0.16 (D), 0.02 (M) mg/mL	Masoko et al. (2005)
Combretum nelsonii	Leaf extracts using: acetone, hexane, dichloromethane, and methanol (successively)	*C. albicans C. neoformans*, *A. fumigates*, *Microsporum canis* and *S. schenckii*	MIC: 0.2 μg/mL *M. canis* and *S. schenckii* were the most susceptible. Antifungal effect confirmed on cutaneous wound healing in immunosuppressed rats	Masoko et al. (2008) Masoko et al. (2010)
Vismia guianensis (AUBL.)	Ethanolic extract of leaves and bark	*S. schenckii ATCC 16345*	MIC: 3.9 μg/mL. Antifungal effect confirmed in a Balb/c mice infection model	Oliveira (2015)

9.8 Conclusions and Future Perspectives

The treatment of sporotrichosis is a challenge for clinical practice. The wide diversity in susceptibility/resistance of the different species and clinical isolates of *S. schenckii* complex to the even more limited antifungals, as well as their frequent toxicity, is a reason to look for new therapeutic alternatives. Different approaches are being evaluated in the search for new antifungals and the use of modern vehicles to improve their target delivery through to new strategies based in novel antifungal combinations, synergistic immunostimulation and other adjuvant modalities, such as photodynamic therapy and the use of medicinal plants. Both combination antifungal therapy and immunomodulatory approaches require detailed study to maximise efficacy and minimise adverse effects. Despite major immunosuppression observed in many patients, vaccines to prevent infection require study, and might be useful for improving therapeutic outcomes. Given the rapid emergence of sporotrichosis in different countries, more effective and safer therapeutic and preventive tools for disease control are urgently needed.

References

Adamu M, Naidoo V, Eloff JN (2012) Some southern African plant species used to treat helminth infections in ethnoveterinary medicine have excellent antifungal activities. BMC Complement Altern Med 12:213

Agüero M, Alvarez S, Luna L et al (2007) Antifungal activity of *Zuccagnia punctata* Cav. Evidences for the mechanism of action. Planta Med 73:1074–1080

Almeida SR (2012) Therapeutic monoclonal antibody for sporotrichosis. Front Microbiol 28 (3):409

Almeida-Paes R, Oliveira MME, Freitas DFS et al (2014) Sporotrichosis in Rio de Janeiro, Brazil: *Sporothrix brasiliensis* is associated with atypical clinical presentations. PLoS Negl Trop Dis 8 (9):e3094

Alvarado-Ramírez E, Torres-Rodríguez JM (2007) In vitro susceptibility of *Sporothrix schenckii* to six antifungal agents determined using three different methods. Antimicrob Agents Chemother 51(7):2420–2423

Apisariyakul A, Vanittanakom N, Buddhasukh D (1995) Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae). J Ethnopharmacol 49:163–169

Arthington-Skaggs BA, Lee-Yang W, Ciblak MA et al (2002) Candidemia Active Surveillance Group. Comparison of visual and spectrophotometric methods of broth microdilution MIC end point determination and evaluation of a sterol quantitation method for in vitro susceptibility testing of fluconazole and itraconazoleagainst trailing and nontrailing Candida isolates. Antimicrob Agents Chemother 46(8):2477–2481

Baginski M, Czub J (2009) Amphotericin B and its new derivatives–mode of action. Curr Drug Metab 10(5):459–469

Barros MBL, Schubach TMP, Galhardo MCG et al (2001) Sporotrichosis: an emergent zoonosis in Rio de Janeiro. Mem Inst Oswaldo Cruz 96:777–779

Barros MBL, Schubach AO, Valle ACF et al (2004) Cat-transmitted sporotrichosis epidemic in Rio de Janeiro, Brazil: description of a series of cases. Clin Infect Dis 38:529–535

Barros MB, Schubach TM, Coll JO et al (2010) Sporotrichosis: development and challenges of an epidemic. Rev Panam Salud Publica 27(6):455–460

Barros MBL, Almeida-Paes R, Schubach AO (2011a) *Sporothrix schenckii* and sporotrichosis. Clin Microbiol Rev 24:633–654

Barros MBL, Schubach AO, de Vasconcellos Carvalhaes de Oliveira R et al (2011b) Treatment of cutaneous sporotrichosis with itraconazole-study of 645 patients. Clin Infect Dis 52(12):e200–e206

Bonifaz A, Fierro L, Saúl A et al (2008) Cutaneous sporotrichosis. Intermittent treatment (pulses) with itraconazole. Eur J Dermatol 18(1):61–64

Borelli C, Schaller M, Niewerth M et al (2008) Modes of action of the new arylguanidine abafungin beyond interference with ergosterol biosynthesis and in vitro activity against medically important fungi. Chemotherapy 54(4):245–259

Bouzabata A, Bazzali O, Cabral C et al (2013) New compounds, chemical composition, antifungal activity and cytotoxicity of the essential oil from *Myrtus nivellei* Batt. & Trab., an endemic species of Central Sahara. J Ethnopharmacol 149(3):613–620

Buissa-Filho R, Puccia R, Marques AF et al (2008) The monoclonal antibody against the major diagnostic antigen of *Paracoccidioides brasiliensis* mediates immuneprotection in infected BALB/c mice challenged intratracheally with the fungus. Infect Immun 76:3321–3328

Bunce PE, Yang L, Chun S et al (2012) Disseminated sporotrichosis in a patient with hairy cell leukemia treated with amphotericin B and posaconazole. Med Mycol 50:197–201

Chapman SW, Pappas P, Kauffmann C et al (2004) Comparative evaluation of the efficacy and safety of two doses of terbinafine (500 and 1000 mg day(-1)) in the treatment of cutaneous or lymphocutaneous sporotrichosis. Mycoses 47:62–68

Chaves AR, Campos MP, Barros MB et al (2013) Treatment abandonment in feline sporotrichosis - study of 147 cases. Zoonoses Public Health 60(2):149–153

Constantinides PP, Chaubal MV, Shorr R (2008) Advances in lipid nanodispersions for parenteral drug delivery and targeting. Adv Drug Deliv Rev 60(6):757–767

Crothers SL, White SD, Ihrke PJ et al (2009) Sporotrichosis: a retrospective evaluation of 23 cases seen in northern California (1987-2007). Vet Dermatol 20(4):249–259

Cruz MC, Santos PO, Barbosa AM Jr et al (2007) Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. J Ethnopharmacol 111:409–412

Czerninski R, Pikovsky A, Gati I, Friedman M et al (2015) Comparison of the efficacy of a novel sustained release clotrimazole varnish and clotrimazole troches for the treatment of oral candidiasis. Clin Oral Investig 19(2):467–473

Daboit TC, Stopiglia CD, von Poser GL et al (2010) Antifungal activity of *Pterocaulon alopecuroides* (Asteraceae) against chromoblastomycosis agents. Mycoses 53:246–250

Dai T, Fuchs BB, Coleman JJ et al (2012) Concepts and principles of photodynamic therapy as an alternative antifungal discovery platform. Front Microbiol 3:120

Danelutte AP, Lago JH, Young MC et al (2003) Antifungal flavanones and prenylated hydroquinones from Piper crassinervium Kunth. Phytochemistry 64:555–559

Darisipudi MN, Allam R, Rupanagudi KV et al (2011) Polyene macrolide antifungal drugs trigger interleukin1β secretion by activating the NLRP3 inflammasome. PLoS One 6(5):e19588

de Almeida JR, Kaihami GH, Jannuzzi GP et al (2015) Therapeutic vaccine using a monoclonal antibody against a 70-kDa glycoprotein in mice infected with highly virulent *Sporothrix schenckii* and *Sporothrix brasiliensis*. Med Mycol 53(1):42–50

de Paula e Silva AC, Oliveira HC, Silva JF et al (2013) Microplate alamarBlue assay for Paracoccidioides susceptibility testing. J Clin Microbiol 51(4):1250–1252

de Sousa MG, Belda W Jr, Spina R et al (2014) Topical application of imiquimod as a treatment for chromoblastomycosis. Clin Infect Dis 58(12):1734–1737

de Souza CML, Nascimento EM, Aparecida MR et al (2011) Gamma radiation effects on *Sporothrix schenckii* yeast cells. Mycopathologia 171:395–401

Delmas G, Park S, Chen ZW et al (2002) Efficacy of orally delivered cochleates containing amphotericin B in a murine model of aspergillosis. Antimicrob Agents Chemother 46 (8):2704–2707

Denning DW, Hope WW (2010) Therapy for fungal diseases: opportunities and priorities. Trends Microbiol 18:195–204

Duan XH, Jiang R, Wen YJ et al (2013) Some 2S albumin from peanut seeds exhibits inhibitory activity against *Aspergillus flavus*. Plant Physiol Biochem 66:84–90

Edwards JE Jr (2012) Fungal cell wall vaccines: an update. J Med Microbiol 61(Pt 7):895–903

Escalante AM, Santecchia CB, López SN et al (2002) Isolation of antifungal saponins from Phytolacca tetramera, an Argentinean species in critic risk. J Ethnopharmacol 82:29–34

Fernandez-Silva F, Capilla J, Mayayo E et al (2012) Efficacy of posaconazole in murine experimental sporotrichosis. Antimicrob Agents Chemother 56(5):2273–2277

Ferreira CP, Galhardo MC, Valle AC (2011) Cryosurgery as adjuvant therapy in cutaneous sporotrichosis. Braz J Infect Dis 15(2):181–183

Flores-García A, Velarde-Félix JS, Garibaldi-Becerra V et al (2015) Recombinant murine IL-12 promotes a protective Th1/cellular response in Mongolian gerbils infected with *Sporothrix schenckii*. J Chemother 27(2):87–93

Francesconi G, Valle ACF, Passos SR et al (2009) Terbinafine (250 mg/day): an effective and safe treatment of cutaneous sporotrichosis. J Eur Acad Dermatol Venereol 23:1273–1276

Francesconi G, Valle ACF, Passos SRL et al (2011) Comparative study of 250 mg/day terbinafine and 100 mg/day itraconazole for the treatment of cutaneous sporotrichosis. Mycopathologia 171:349–354

Franco DD, Nascimento RC, Ferreira KS et al (2012) Antibodies against *Sporothrix schenckii* enhance TNF-a production and killing by macrophages. Scand J Immunol 75:142–146

Freitas DF, de Siqueira Hoagland B, do Valle AC et al (2012) Sporotrichosis in HIV-Infected patients: report of 21 cases of endemic sporotrichosis in Rio de Janeiro, Brazil. Med Mycol 50:170–178

Freitas DFS, do Valle ACF, Silva MBT et al (2014) Sporotrichosis: an emerging neglected opportunistic infection in HIV-infected patients in Rio de Janeiro, Brazil. PLoS Negl Trop Dis 8(8), e3110

Gaitán I, Paz AM, Zacchino SA et al (2011) Subcutaneous antifungal screening of Latin American plant extracts against *Sporothrix schenckii* and *Fonsecaea pedrosoi*. Pharm Biol 49 (9):907–919

Gilaberte Y, Aspiroz C, Alejandre MC et al (2014) Cutaneous sporotrichosis treated with photodynamic therapy: an in vitro and in vivo study. Photomed Laser Surg 32(1):54–57

Goldman DL, Casadevall A, Zuckier LS (1997) Pharmacokinetics and biodistribution of a monoclonal antibody to *Cryptococcus neoformans* capsular polysaccharide antigen in a rat model of cryptococcal meningitis: implications for passive immunotherapy. J Med Vet Mycol 35(4):271–278

Goldstein AS, Lukaynov AN (1997) Formation of high axial ratio microstructures from natural and synthetic sphingo lipids. Chem Phys Lipids 88:21–36

Greene CE, Calpin J (2012) Antimicrobial drug formulary. In: Greene CE (ed) Infectious diseases in dog and cat, 4th edn. Saunders Elsevier, St. Louis, pp 1207–1320

Gremião ID, Pereira SA, Rodrigues AM et al (2006) Tratamento cirúrgico associado à terapia antifúngica convencional na esporotricose felina. Acta Sci Vet 34(2):221–223

Gremião ID, Schubach TM, Pereira SA et al (2009) Intralesional amphotericin B in a cat with refractory localised sporotrichosis. J Feline Med Surg 11(8):720–723

Gremião ID, Schubach TM, Pereira SA et al (2011) Treatment of refractory feline sporotrichosis with a combination of intralesional amphotericin B and oral itraconazole. Aust Vet J 89 (9):346–351

Gremião ID, Menezes RC, Schubach TMP et al (2015) Feline sporotrichosis: epidemiological and clinical aspects. Med Mycol 53(1):15–21

Guterres KA, de Matos CB, Osório Lda G et al (2014) The use of (1-3) β-glucan along with itraconazole against canine refractory sporotrichosis. Mycopathologia 177(3–4):217–221

Gutierrez-Galhardo MC, Oliveira RMZ, Valle ACF et al (2008) Molecular epidemiology and antifungal susceptibility patterns of *Sporothrix schenckii* isolates from a cat-transmitted epidemic of sporotrichosis in Rio de Janeiro, Brazil. Med Mycol 46:141–151

Heidrich D, Stopiglia CD, Senter L et al (2011) Successful treatment of terbinafine in a case of sporotrichosis. An Bras Dermatol 86(4 Suppl 1):S182–S185

Heit MC, Riviere J (1995) Antifungal and antiviral drugs. In: Adams R (ed) Veterinary pharmacology and therapeutics, 7th edn. Iowa State University Press, Ames, pp 855–885

Hirano M, Watanabe K, Murakami M et al (2006) A case of feline sporotrichosis. J Vet Med Sci 68 (3):283–284

Honse CO, Rodrigues AM, Gremião ID et al (2010) Use of local hyperthermia to treat sporotrichosis in a cat. Vet Rec 166(7):208–209

Italia JL, Yahya MM, Singh D (2009) Biodegradable nanoparticles improve oral bioavailability of amphotericin B and show reduced nephrotoxicity compared to intravenous Fungizone®. Pharm Res 26(6):1324–1331

Johann S, Pizzolatti MG, Donnici CL et al (2007) Antifungal properties of plants used in Brazilian traditional medicine against clinically relevant fungal pathogens. Braz J Microbiol 38:632–637

Johann S, Cota BB, Souza-Fagundes EM et al (2009) Antifungal activities of compounds isolated from *Piper abutiloides* Kunth. Mycoses 52(6):499–506

Jung SH, Lim DH, Jung SH et al (2009) Amphotericin B-entrapping lipid nanoparticles and their *in vitro* and *in vivo* characteristics. Eur J Pharm Sci 37:313–320

Kalaskar PS, Karande VV, Bannalikar AS et al (2012) Antifungal activity of leaves of mangroves plant acanthus licifolius against *Aspergillus fumigatus*. Indian J Pharm Sci 74(6):575–579

Katz HI (1999) Drug interactions of the newer oral antifungal agents. Br J Dermatol 141(Suppl 56):26–32

Kauffman CA, Bustamante B, Chapman SW et al (2007) Clinical Practice Guidelines for the management of sporotrichosis. 2007 update by the Infectious Disease Society of America. Clin Infect Dis 45:1255–1265

Kohler LM, Monteiro PC, Hahn RC et al (2004) In vitro susceptibilities of isolates of *Sporothrix schenckii* to itraconazole and terbinafine. J Clin Microbiol 42(9):4319–4320

Krenova Z, Pavelka Z, Lokaj P et al (2010) Successful treatment of life-threatening *Candida peritonitis* in a child with abdominal non-Hodgkin lymphoma using Efungumab and amphotericin B colloid dispersion. J Pediatr Hematol Oncol 32(2):128–130

Kullberg BJ, van de Veerdonk F, Netea MG (2014) Immunotherapy: a potential adjunctive treatment for fungal infection. Curr Opin Infect Dis 27(6):511–516

Kwon-Chung KJ, Bennett JE (1992) Medical mycology, 2nd edn. Lea & Febiger, Philadelphia, pp 707–729

Lacaz CS (2002) Esporotricose e outras micoses gomosas. In: Lacaz CS, Porto E, Martins JEC, Heins-vaccari EM, Melo NT (eds) Tratado de Micologia Médica Lacaz, 9th edn. Sarvier, São Paulo, pp 479–497

Larsen RA, Pappas PG, Perfect J et al (2005) Phase I evaluation of the safety and pharmacokinetics of murine-derived anticryptococcal antibody 18B7 in subjects with treated cryptococcal meningitis. Antimicrob Agents Chemother 49(3):952–958

Laverdiere M, Bow EJ, Rotstein C et al (2014) Therapeutic drug monitoring for triazoles: a needs assessment review and recommendations from a Canadian perspective. Can J Infect Dis Med Microbiol 25(6):327–343

Lee KC, Carlson PA (1999) Protection of deca-peptide from proteolytic cleavage by lipidation and self assembly into high axial ratio micro structures. Langmuir 15:550–5508

Lee KC, Lukaynov AN (1998) Formation of high axial ratio microstructures from peptides modified with glutamic acid dialkyl amides. Biochim Biophys Acta 1371:168–184

Lloret A, Hartmann K, Pennisi MG et al (2013) Sporotrichosis in cats: ABCD guidelines on prevention and management. J Feline Med Surg 15:619–623

Lopez-Berestein G, Hopfer RL, Mehta R et al (1984) Liposome-encapsulated amphotericin B for the treatment of disseminated candidiasis in neutropenic mice. J Infect Dis 150:278–283

Lyon JP, Silva Azevedo C, Moreira LM et al (2011) Photodynamic antifungal therapy against chromoblastomycosis. Mycopathologia 172:293–297

Madrid IM, Xavier MO, Mattei AS et al (2007) Esporotricose óssea e cutânea em um canino. Braz J Vet Res Anim Sci 44(6):441–443

Malheiros A, Cechinel Filho V, Schmitt CB et al (2005) Antifungal activity of drimane sesquiterpenes from Drimys brasiliensis using bioassay guided fractionation. J Pharm Pharm Sci 8:335–339

Malik R, Krockenberger MB, O'Brien CR (2009) Intra-lesional amphotericin B—worth a try, maybe for lots of things, but we need more data! J Feline Med Surg 11:621–623

Mannino R, Gold-Foserite S, Kheiri MT et al (1998) Targeting immune response induction with cochleate and liposomes based vaccines. Adv Drug Deliv Rev 32:237–287

Maregesi SM, Pieters L, Ngassapa OD et al (2008) Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. J Ethnopharmacol 119:58–66

Marimon R, Serena C, Gene J et al (2008) In vitro antifungal susceptibilities of five species of *Sporothrix*. Antimicrob Agents Chemother 52(2):732–734

Masoko P, Picard J, Eloff JN (2005) Antifungal activities of six South African Terminalia species (Combretaceae). J Ethnopharmacol 99(2):301–308

Masoko P, Mdee LK, Mampuru LJ et al (2008) Biological activity of two related triterpenes isolated from *Combretum nelsonii* (Combretaceae) leaves. Nat Prod Res 22(12):1074–1084

Masoko P, Picard J, Howard RL et al (2010) In vivo antifungal effect of *Combretum* and *Terminalia* species extracts on cutaneous wound healing in immunosuppressed rats. Pharm Biol 48(6):621–632

Meinerz AR, Nascente PS, Schuch LF et al (2007) Suscetibilidade in vitro de isolados de *Sporothrix schenckii* frente à terbinafina e itraconazol. Rev Soc Bras Med Trop 40:60–62

Merck (2014) http://www.merck.com/product/usa/pi_circulars/n/noxafil/ noxafil_pi.pdf. Accessed 25 Aug 2014

Mesa-Arango AC, Scorzoni L, Zaragoza O (2012) It only takes one to do many jobs: Amphotericin B as antifungal and immunomodulatory drug. Front Microbiol 3:286

Micromedex: The Complete Drug Reference (2014) http://www-micromedexsolutions-com.ez68.periodicos.capes.gov.br/micromedex2/ librarian/. Accessed 28 Aug 2014

Mihu MR, Pattabhi R, Nosanchuk JD (2014) The impact of antifungals on toll-like receptors. Front Microbiol 5:1–5

Moen MD, Lyseng-Williamson KA, Scott LJ (2009) Liposomal amphotericin B: a review of its use as empirical therapy in febrile neutropenia and in the treatment of invasive fungal infections. Drugs 69(3):361–392

Mokoka TA, McGaw LJ, Eloff JN (2010) Antifungal efficacy of ten selected South African plant species against *Cryptococcus neoformans*. Pharm Biol 48(4):397–404

Mukherjee S, Lee S, Mukherjee J et al (1994) Monoclonal antibodies to *Cryptococcus neoformans* capsular polysaccharide modify the course of intravenous infection in mice. Infect Immun 62 (3):1079–1088

Nanjappa SG, Klein BS (2014) Vaccine immunity against fungal infections. Curr Opin Immunol 28:27–33

Nascimento RC, Espíndola NM, Castro RA et al (2008) Passive immunization with monoclonal antibody against a 70-kDa putative adhesin of *Sporothrix schenckii* induces protection in murine sporotrichosis. Eur J Immunol 38(11):3080–3089

National Committee for Clinical Laboratory Standards (NCCLS) (1997) Reference method for broth dilution antifungal susceptibility testing of yeast. Approved standard M27-A. 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, USA

National Committee for Clinical Laboratory Standards (NCCLS) (2002) Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A. 940 West Valley Road, Suite 1400, Wayne, Pennsylvania, USA

Nowosielski M, Hoffmann M, Wyrwicz LS et al (2011) Detailed mechanism of squalene epoxidase inhibition by terbinafine. J Chem Inf Model 51(2):455–462

Oliveira AH (2015) Avaliação das atividades anti-inflamatória e Antifúngica dos extratos de *Vismia guianensis (Aubl.)* Choisy na infecção induzida por *Sporothrix schenckii*. Tese doutoral em Biociências e Biotecnologia Aplicadas à Farmácia, da Faculdade de Ciências Farmacêuticas de Araraquara, UNESP

Otang WM, Grierson DS, Ndip RN (2012) Phytochemical studies and antioxidant activity of two South African medicinal plants traditionally used for the management of opportunistic fungal infections in HIV/AIDS patients. BMC Complement Altern Med 12:43

Ottonelli CD, Magagnin CM, Castrillón MR et al (2014) Antifungal susceptibilities and identification of species of the *Sporothrix schenckii* complex isolated in Brazil. Med Mycol 52 (1):56–64

Pachl J, Svoboda P, Jacobs F, Vandewoude K, van der Hoven B, Spronk P et al (2006) A randomized, blinded, multicenter trial of lipid-associated amphotericin B alone versus in combination with an antibody-based inhibitor of heat shock protein 90 in patients with invasive candidiasis. Clin Infect Dis 42(10):1404–1413

Papahadjopoulos D, Wilschut J (1979) Calcium induced fusion of phospholipids vehicles. Nature 281:690–692

Patel PA, Patravale VB (2011) AmbiOnp: solid lipid nanoparticles of amphotericin B for oral administration. J Biomed Nanotechnol 7(5):632–639

Pereira SA, Schubach TM, Gremião ID et al (2009) Aspectos terapêuticos da esporotricose felina. Acta Sci Vet 37(4):331–341

Pereira SA, Passos SR, Silva JN et al (2010) Response to azolic antifungal agents for treating feline sporotrichosis. Vet Rec 166(10):290–294

Pereira SA, Gremião ID, Kitada AA et al (2014) The epidemiological scenario of feline sporotrichosis in Rio de Janeiro, State of Rio de Janeiro, Brazil. Rev Soc Bras Med Trop 47 (3):392–393

Perlin DS (2004) Amphotericin B cochleates: a vehicle for oral delivery. Curr Opin Investig Drugs 5(2):198–201

Portuondo DL, Ferreira LS, Urbaczek AC et al (2015a) Adjuvants and delivery systems for antifungal vaccines: current state and future developments. Med Mycol 53(1):69–89

Portuondo DL (2015b) Desenvolvimento e avaliação de candidatos vacinais a base de proteínas da superfície celular de Sporothrix schenckii. Tese doutoral em Biociências e Biotecnologia Aplicadas à Farmácia, da Faculdade de Ciências Farmacêuticas de Araraquara, UNESP

Price RR, Patchan M (1991) Controlled release from cylindrical microstructures. J Microencapsul 8:301–306

Qiao J, Li R, Ding Y, Fang H (2010) Photodynamic therapy in the treatment of superficial mycoses: an evidence based evaluation. Mycopathologia 170:339–343

Rai M, Mares D (2003) Plant-derived antimycotics. Food Products Press, New York, p 587

Rao R, Squillante E, Kim KH (2007) Lipid-based cochleates: a promising formulation platform for oral and parenteral delivery of therapeutic agents. Crit Rev Ther Drug Carrier Syst 24(1):41–61

Reis EG, Gremião ID, Kitada AA et al (2012a) Potassium iodide capsule treatment of feline sporotrichosis. J Feline Med Surg 14(6):399–404

Reis EG, Kitada AA, Carvalho BW et al (2012b). Treatment of refractory feline sporotrichosis with potassium iodide capsule. XVIII International Congress for Tropical Medicine and Malaria, Rio de Janeiro, Brazil

Rex JH, Nelson PW, Paetznick VL et al (1998) Optimising the correlation between results of testing in vitro and therapeutic outcome in vivo for fluconazole by testing critical isolates in a murine model of invasive candidiasis. J Clin Microbiol 42:129–134

Rippon JW (1988) Medical mycology: the pathogenic fungi and the pathogenic actinomycetes, 3rd edn. WB Saunders, Philadelphia, pp 325–352

Rocha RFDB (2014) Tratamento da esporotricose felina refratária com a associação de iodeto de potássio e itraconazol oral. Dissertação apresentada ao Curso de pós-graduação em Pesquisa

Clínica em Doenças Infecciosas do Instituto de Pesquisa Clínica Evandro Chagas para obtenção do grau de Mestre em Ciências
Rodrigues AM, de Hoog GS, de Cássia Pires D et al (2014) Genetic diversity and antifungal susceptibility profiles in causative agents of sporotrichosis. BMC Infect Dis 14:219
Rojas R, Bustamante B, Bauer J et al (2003) Antimicrobial activity of selected Peruvian medicinal plants. J Ethnopharmacol 88(2-3):199–204
Rosa AC, Scroferneker ML, Vettorato R et al (2005) Epidemiology of sporotrichosis: a study of 304 cases in Brazil. J Am Acad Dermatol 52:451–459
Rosser E, Dunstan R (2006) Sporotrichosis. In: Greene CE (ed) Infectious diseases in dog and cat, 3rd edn. Saunders Elsevier, St. Louis, MO, pp 608–612
Rossi CN, Odaguiri J, Larsson CE (2013) Retrospective assessment of the treatment of sporotrichosis in cats and dogs using itraconazole. Acta Sci Vet 41:1112
Saag MS, Dismukes WE (1988) Azole antifungal agents: emphasis on new triazoles. Antimicrob Agents Chemother 32(1):1–8
Santangelo R, Paderu P, Delmas G et al (2000) Efficacy of oral cochleate-amphotericin B in a mouse model of systemic candidiasis. Antimicrob Agents Chemother 44(9):2356–2360
Scheinfeld N (2007) A review of the new antifungals: posaconazole, micafungin, and anidulafungin. J Drugs Dermatol 6:1249–1251
Schubach TM, Schubach A, Okamoto T et al (2004) Evaluation of an epidemic of sporotrichosis in cats: 347 cases (1998-2001). J Am Vet Med Assoc 224(10):1623–1629
Schubach TM, Schubach A, Okamoto T et al (2006) Canine sporotrichosis in Rio de Janeiro, Brazil: clinical presentation, laboratory diagnosis and therapeutic response in 44 cases (1998-2003). Med Mycol 44(1):87–92
Schubach TM, Menezes RC, Wanke B (2012) Sporotrichosis. In: Greene CE (ed) Infectious diseases of the dog and cat, 4th edn. Saunders Elsevier, Philadelphia, pp 645–650
Seibold M, Tintelnot K (2003) Susceptibility testing of fungi - current status and open questions. Prog Drug Research 191–241
Silveira CP, Torres-Rodriguez JM, Alvarado-Ramirez E et al (2009) MICs and minimum fungicidal concentrations of amphotericin B, itraconazole, posaconazole and terbinafine in *Sporothrix schenckii*. J Med Microbiol 58(Pt 12):1607–1610
Singh DN, Verma N, Raghuwanshi S et al (2008) Antifungal activity of *Agapanthus africanus* extractives. Fitoterapia 79(4):298–300
Steinbach WJ, Stevens DA (2003) Review of newer antifungal and immunomodulatory strategies for invasive aspergillosis. Clin Infect Dis 37(Suppl 3):S157–S187
Sterling JB, Heymann WR (2000) Potassium iodide in dermatology: a 19th century drug for the 21st century-uses, pharmacology, adverse effects, and contraindications. J Am Acad Dermatol 43:691–697
Stevens DA (1998) Combination immunotherapy and antifungal chemotherapy. Clin Infect Dis 26 (6):1266–1269
St-Germain G (2001) Impact of endpoint definition on the outcome of antifungal susceptibility tests with Candida species: 24- versus 48-h incubation and 50 versus 80% reduction in growth. Mycoses 44:37–45
Stopiglia CD, da Rocha VD, de Carvalho MG et al (2011) Antifungal activity of *Pterocaulon* species (Asteraceae) against *Sporothrix schenckii*. J Mycol Med 21(3):169–172
Svetaz L, Zuljan F, Derita M et al (2010) Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. J Ethnopharmacol 127:137–158
Syed UM, Woo AF, Plakogiannis F et al (2008) Cochleates bridged by drug molecules. Int J Pharm 363(1–2):118–125
Toledo MS, Tagliari L, Suzuki E et al (2010) Effect of anti-glycosphingolipid monoclonal antibodies in pathogenic fungal growth and differentiation. Characterization of monoclonal antibody MEST-3 directed to Manpalpha1– > 3Manpalpha1– > 2IPC. BMC Microbiol 10:47

Torrado JJ, Espada R, Ballesteros MP et al (2008) Amphotericin B formulations and drug targeting. J Pharm Sci 97(7):2405–2425

Torres-Mendoza BM, Vazquez-Valls E, Gonzalez-Mendoza A (1997) Effect of potassium iodide on the immune response in the sporotrichosis. Rev Iberoam Micol 14:98–100

Valle ACF, Gutierrez-Galhardo MC (2012) Esporotricose. In: Tavares W, Marinho LAC (eds) Rotinas de diagnóstico e tratamento das doenças infecciosas e parasitárias, 3rd edn. Atheneu, São Paulo, pp 332–336

Van de Ven H, Paulussen C, Feijens PB et al (2012) PLGA nanoparticles and nanosuspensions with amphotericin B: potent in vitro and in vivo alternatives to Fungizone and Am Bisome. J Control Release 161(3):795–803

Warrilow AG, Parker JE, Kelly DE et al (2013) Azole affinity of sterol 14α-demethylase (CYP51) enzymes from *Candida albicans* and *Homo sapiens*. Antimicrob Agents Chemother 57 (3):1352–1360

Wen C, Guo W, Chen X (2014) Purification and identification of a novel antifungal protein secreted by *Penicillium citrinum* from the Southwest Indian Ocean. J Microbiol Biotechnol 24(10):1337–1345

Whittemore JC, Webb CB (2007) Successful treatment of nasal sporotrichosis in a dog. Can Vet J 48(4):411–414

Wong-Beringer A, Jacobs RA, Guglielmo BJ (1998) Lipid formulations of amphotericin B: clinical efficacy and toxicities. Clin Infect Dis 27:603–618

Woolley DW (1944) Some biological effects produced by benzimidazole and their reversal by purines. J Biol Chem 152:225–232

Xander P, Vigna AF, Feitosa LDS et al (2007) A surface75-kDa protein with acid phosphatase activity recognized by monoclonal anti-bodies that inhibit *Paracoccidioides brasiliensis* growth. Microb Infect 9:1484–1492

Zarai Z, Kadri A, Ben Chobba I et al (2011) The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of *Marrubium vulgare* L essential oil grown in Tunisia. Lipids Health Dis 10:161

Zarif L, Mannino R (2000) Cochleates: new lipid based vehicles for gene deliveries, cancer gene therapy, vol 465, Advances in experimental medicine and biology. Kluwer, New York, pp 83–94

Zarif L, Graybill JR, Perlin D et al (2000) Antifungal activity of amphotericin B cochleates against *Candida albicans* infection in a mouse model. Antimicrob Agents Chemother 44 (6):1463–1469

Zhang HB, Jia CK, Xi HJ et al (2011) Specific inhibition of *Candida albicans* growth *in vitro* by antibodies from experimental *Candida keratitis* mice. Exp Eye Res 93:50–58

Zhang G, Zhang Y, Qin J et al (2013) Antifungal metabolites produced by *Chaetomium globosum* No.04, an endophytic fungus isolated from *Ginkgo biloba*. Indian J Microbiol 53(2):175–180

Zotchev SB (2003) Polyene macrolide antibiotics and their applications in human therapy. Curr Med Chem 10(3):211–223

Index

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