



Mycologic Forum

Cell wall proteins of *Sporothrix schenckii* as immunoprotective agents



Carlos A. Alba-Fierro^a, Armando Pérez-Torres^b, Everardo López-Romero^c,
Mayra Cuéllar-Cruz^c, Estela Ruiz-Baca^{a,*}

^a Facultad de Ciencias Químicas, Universidad Juárez del Estado de Durango, México

^b Facultad de Medicina, Universidad Nacional Autónoma de México DF, México

^c Departamento de Biología, División de Ciencias Naturales y Exactas, Campus Guanajuato, Universidad de Guanajuato, Guanajuato, México

ARTICLE INFO

Article history:

Received 17 August 2013

Accepted 26 September 2013

Available online 17 November 2013

Keywords:

Cell wall antigens

Cellular and humoral responses

Immunoprotection

Sporothrix schenckii

Sporotrichosis

ABSTRACT

Sporothrix schenckii is the etiological agent of sporotrichosis, an endemic subcutaneous mycosis in Latin America. Cell wall (CW) proteins located on the cell surface are inducers of cellular and humoral immune responses, potential candidates for diagnosis purposes and to generate vaccines to prevent fungal infections. This mini-review emphasizes the potential use of *S. schenckii* CW proteins as protective and therapeutic immune response inducers against sporotrichosis. A number of pathogenic fungi display CW components that have been characterized as inducers of protective cellular and humoral immune responses against the whole pathogen from which they were originally purified. The isolation and characterization of immunodominant protein components of the CW of *S. schenckii* have become relevant because of their potential in the development of protective and therapeutic immune responses against sporotrichosis.

This manuscript is part of the series of works presented at the “V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi” (Oaxaca, Mexico, 2012).

© 2013 Revista Iberoamericana de Micología. Published by Elsevier España, S.L. All rights reserved.

Proteínas de la pared celular de *Sporothrix schenckii* como moléculas inmunoprotectoras

RESUMEN

Sporothrix schenckii es el agente etiológico de la esporotricosis, una micosis subcutánea endémica en América Latina. Las proteínas de la pared celular (PC), localizadas en la superficie celular, inducen respuestas de inmunidad celular y humoral, y son candidatas potenciales tanto para objetivos diagnósticos como para la generación de vacunas en la prevención de las infecciones fúngicas. En la presente revisión se destaca el uso potencial de las proteínas de la PC de *S. schenckii* como inductoras de una respuesta inmunitaria protectora y terapéutica frente a la esporotricosis. Muchos de los hongos patógenos presentan componentes de la pared celular que se han caracterizado como inductores de respuestas inmunológicas celulares y humorales protectoras frente al patógeno a partir del cual se obtienen. El aislamiento y caracterización de los componentes proteicos inmunodominantes de la pared celular de *S. schenckii* llegan a ser pertinentes para su uso como inductores del desarrollo de respuestas inmunitarias protectoras y terapéuticas frente a la esporotricosis.

Este artículo forma parte de una serie de estudios presentados en el «V International Workshop: Molecular genetic approaches to the study of human pathogenic fungi» (Oaxaca, México, 2012).

© 2013 Revista Iberoamericana de Micología. Publicado por Elsevier España, S.L. Todos los derechos reservados.

Sporothrix schenckii is a dimorphic fungus and the etiological agent of sporotrichosis.²⁵ Natural infection and disease occur after

the mycelial form of the fungus penetrates the host through skin abrasions produced by fungal-contaminated plants or animals (or, more rarely, after inhalation) and converts into the yeast morphotype. Acute or chronic cutaneous and lymphocutaneous lesions are the most common clinical manifestations of sporotrichosis.⁵⁰ Frequently, the occurrence of systemic and disseminated cutaneous

* Corresponding author.

E-mail addresses: erb750@hotmail.com, eruiz@ujed.mx (E. Ruiz-Baca).

forms is associated with immunosuppression.³⁹ Hence, the efficiency of the host immune responses, along with the virulence and pathogenicity of the strain, determines the extent of fungal invasion.^{26,45} Sporotrichosis has been considered as an occupational disease since it commonly affects gardeners, florists, horticulturists, etc. These individuals are exposed to plants and soil that constitute the ecological niche of the pathogen.^{25,36} Sporotrichosis has a cosmopolitan distribution and is especially endemic in Latin American areas with tropical and subtropical climates.^{25,38} However, *S. schenckii* is not the only etiologic agent of sporotrichosis. Other members of the *Sporothrix* species complex, such as *S. schenckii sensu stricto*, *Sporothrix brasiliensis*, *Sporothrix globosa*, *Sporothrix mexicana*, *Sporothrix luriei*, and *Sporothrix albicans*,^{25,27,28} may also be relevant in this context. However, until now, only *S. schenckii sensu stricto*, *S. brasiliensis*, and *S. globosa* have sparked medical interest. Furthermore, other three *Sporothrix* environmental species, namely, *Sporothrix stylites*, *Sporothrix humicola*, and *Sporothrix lignivora*, have recently been described.¹¹

Adhesion of the fungus to host cells plays a central role in pathogenesis.²⁰ Due to its location, composition, and immunogenicity, the CW is the major fungal structure involved in the interaction with the host, and its immunogenicity is mediated by a variety of molecules that includes glycoproteins, polysaccharides, lipids, and pigments.³⁴ Interestingly, some of these CW components have also the potential to modify the course of the fungal disease in favor of the host.³¹ Various *S. schenckii* CW and secretory molecules are highly immunogenic and thus are potential targets for the development of vaccines and serological tests for the diagnosis and treatment of sporotrichosis.^{40–42,48}

Cell wall immunogenicity

The CW of *S. schenckii* contains alkali-soluble and -insoluble glycans that are found in similar proportions in its two morphotype phases.^{25,35,41} One of these components is the peptide rhamnomannan, a polymer whose chains are constituted of α -1,6-linked mannosyl and α -D glucuronic acid units.^{18,49} This macromolecule has been isolated from the CW of yeast-like cells of *S. schenckii* and has a polysaccharide composition of D-mannose (50%), L-rhamnose (33%), and galactose (1%), as well as a peptide fraction of nearly 16%.²⁴ The peptide-rhamnomannan can be separated into two fractions depending on its affinity to the lectin concanavalin A (Con-A). The fraction that binds Con-A is relevant to the diagnosis of sporotrichosis in humans, as the peptide is recognized by 100% of sera of patients suffering cutaneous sporotrichosis.³³ In other clinical forms, the same fraction demonstrates 90% of sensitivity and 86% of global efficiency.¹ These percentages may vary depending on the fractions extracted from different strains.² The fraction that binds Con-A is also useful for the diagnosis of feline sporotrichosis, with high sensitivity and specificity.¹⁴ These results suggest that the identification of the immunoreactive components of the CW can guide the design of tools for a highly sensitive and specific diagnosis of this mycosis in its different clinical forms.

Two glycoproteins of 60 and 70 kDa, named as Gp60 and Gp70, respectively, have been demonstrated to be the most immunogenic components of *S. schenckii* CW.⁴¹ Two-dimensional immunoblots have revealed that Gp60, which has an isoelectric point between 4.5 and 5.1, is present only in the yeast morphotype. More recently, it has been demonstrated that the three clinically relevant species (i.e., *S. schenckii sensu stricto*, *S. brasiliensis*, and *S. globosa*) of the *Sporothrix* complex secrete a 60 kDa protein that is recognized only by sera of mice infected with the most virulent strains.¹³ In contrast, Gp70, which has an isoelectric point of 4.1 and contains approximately 5.7% of its molecular mass of N-linked glycans, is present in both fungal morphotypes. This glycoprotein is potentially involved

in adhesion during host–pathogen interaction since incubation of yeast-like cells of *S. schenckii* with anti-Gp70 heteroclonal antibodies decreases the fungal ability to adhere to components of the dermal extracellular matrix from mouse tails.⁴² Studies using a soluble antigen mixture derived from *S. schenckii* and sera from patients with extracutaneous sporotrichosis have revealed that these patients present elevated titers of antibodies that recognize between 15 and 20 proteins with molecular weights ranging from 22 to 70 kDa. However, recognition was reduced to 8–10 proteins when sera from patients with cutaneous sporotrichosis were used. Notably, the immunodominant antigens were the 40 and 70 kDa proteins identified with all serum samples.⁴³

The relevance of Gp60 and Gp70 expression in both morphotypes of *S. schenckii* in the context of natural and experimental infections has not yet investigated. Likewise, the relationship of these glycoproteins to the host immune response and the evolution of sporotrichosis are unknown. A better understanding of these aspects would facilitate the design of more effective immunoprotective strategies as Gp60 and Gp70 are the most immunogenic glycoproteins in *S. schenckii* and because their presence in the CW is related to dimorphic transition, which is a relevant attribute of fungal pathogenicity.

Immunity against cell wall components

The pathogenicity and virulence of microorganisms trigger a variety of innate and adaptive immune responses in the host that, under optimal conditions, delay the spread of the invading microorganism and may lead to its eradication. In fungi, CW components have already been proven to induce protective responses in several mycoses such as blastomycosis, histoplasmosis, paracoccidioidomycosis, coccidioidomycosis, and candidiasis. In all cases, cellular and humoral responses are important to achieve a protective state.³⁴ To date, there are few studies addressing similar strategies for protection against sporotrichosis.

Cellular immunity

The cellular immune response, or Th1 response, is characterized by an increase in the levels of cytokines such as IFN- γ , which activates effector cells of the immune system, and IL-12, which induces increased production of IFN- γ and activates CD8⁺ lymphocytes. The relevance of this response against *S. schenckii* has been demonstrated by the higher susceptibility to this fungal infection of athymic (nu/nu) versus normal mice.⁴⁴ Furthermore, when athymic mice are reconstituted with thymocytes, the animals acquire higher resistance compared to control groups.¹² By contrast, mice injected with a peptide fraction of *S. schenckii* display a delayed hypersensitivity response, although spleen lymphocytes from mice systemically infected with this fungus are not able to proliferate *in vitro* when challenged with the same antigen.⁵ Further evidence supporting the relevance of the cellular immune response against this fungus is the protection achieved after reconstitution with lymph node cells from pre-immunized mice to BALB/c mice infected subcutaneously, which display a lower fungal burden compared to control group mice. Moreover, the protective or anti-fungal effect is abrogated when, prior to the passive transfer, primed cells are incubated with monoclonal antibodies specific for CD4 lymphocytes and macrophages. Incubation of lymph node cells with *S. schenckii* stimulates the production of IFN- γ , which is a potent inducer of fungicidal activity in macrophages.⁴⁶ These studies highlight the protective importance of cellular immunity against fungal infection, and other studies using more specific cell wall antigens also confirm this view. Thus, the mannose protein MP65 from the CW of *Candida albicans* has been demonstrated to exert a potent lymphoproliferative effect,¹⁷ and the 70 kDa

heat-shock protein, which is also present on the surface of this organism, is a potent inducer of cellular immunity via the stimulation of IFN- γ production.³

In the same line, the 120 kDa protein from the CW of *Blastomyces dermatitidis* induces strong cellular and humoral responses in humans and dogs with a blastomycosis infection.²¹ Similarly, HIS-62 and HIS-80, two CW and membrane proteins from *Histoplasma capsulatum*, are the main inducers of cellular immune response in patients with histoplasmosis.¹⁶ Also, a 60 kDa heat shock protein isolated from *Paracoccidioides brasiliensis* induces protection against experimental lung infections caused by this fungus. This protective response is most likely mediated by the production of IFN- γ and IL-12.⁷

Humoral immunity

Th2 response is characterized by the presence of IL-4, which favors and maintains the development of subpopulations of Th2 lymphocytes, and IL-10, which inhibits the synthesis of IL-12. Additionally, antibody production is another distinctive feature of the Th2 response. Although humoral immune response to *S. schenckii* is poorly studied, it has been identified by ELISA and western blot analyses in patients with sporotrichosis.⁴³ Interestingly, mice with experimental sporotrichosis display Th1 response during the entire infective period and develop a Th2 response only during the most advanced phases.²⁶ In this sense, sera from BALB/c mice infected with *S. schenckii* exhibit a Th2 response that is represented by IgG1 and IgG3 isotypes specific for a soluble 70 kDa protein.²⁹ Recently, similar results have been obtained using other virulent strains of the *Sporothrix* complex to infect BALB/c mice which produce antibodies specific for a 60 kDa secretory protein. This finding suggests that this protein might be involved in fungal virulence¹³ and is probably related to the Gp60 protein from the CW, as previously described by Ruiz-Baca et al.⁴¹ Infections with other pathogenic fungi have also demonstrated that the host can manifest a significant humoral response. CW proteins from *C. albicans* in a 20–260 kDa range, the 120 kDa glycoprotein from *Coccidioides immitis*, and the WI-1 protein from *B. dermatitidis* are all efficient inducers of humoral response.^{6,22,34}

Suitability of cell wall components as immunoprotective agents

It is well established that subcutaneous inoculation of *S. schenckii* yeast-like cells into BALB/c mice prior to a subsequent lethal intravenous infection with this same fungus, results in a protective response against infection and a concomitantly lower fungal burden. This protection may be induced in athymic mice once they are implanted with lymph node cells from immunized mice.⁴⁶

In contrast with other dimorphic fungi such as *H. capsulatum*, *P. brasiliensis*, *Coccidioides* spp., *B. dermatitidis*, or *C. albicans*, there is little information regarding specific CW molecules from *S. schenckii* with the ability to modify the course of the infection. Therefore, the immunogenic Gp70 and Gp60 proteins of this pathogen are attractive research targets. Gp70 has adhesive properties and hence it could be defined as virulence attribute. This characteristic is suggested by the fact that pre-incubation of *S. schenckii* cells with anti-Gp70 antibodies decreases fungal adhesion to the extracellular matrix of the mouse tail dermis. It has been suggested that this protein may be a potential target for treating this infection.⁴² The injection of monoclonal antibodies directed against a 70 kDa protein of *S. schenckii* induces a passive immune state that is effective in BALB/c mice subsequently infected with yeast-like cells by intraperitoneal via.³⁰ The relevance of humoral immunity against *S. schenckii* infection and protection of the host is demonstrated by the fact that similar results can be obtained

using monoclonal antibodies specific for this fungus. However, the implicated mechanisms with the use of monoclonal antibodies during mycotic infections have not been elucidated. It is possible that these antibodies function as opsonins and facilitate phagocytosis of the fungus,¹⁰ as it has been suggested in BALB/c mice after the use of monoclonal antibodies specific to a 70 kDa (Gp70) cellular surface glycoprotein of *P. brasiliensis*, which alter the pulmonary granuloma development. The Gp60 glycoprotein from the CW of *S. schenckii* is one of the immunodominant proteins and is a possible fungal virulence factor. Consequently, Gp60 is considered an ideal candidate to be used as an inducer of potent cellular and humoral responses that may result in immune protection.^{13,41} This assumption is based on the immune protection obtained after immunization with the 60 kDa heat-shock proteins (Hsp60) isolated from the CW of *H. capsulatum* or *P. brasiliensis*, during experimental pulmonary histoplasmosis¹⁵ and experimental paracoccidioidomycosis in BALB/c mice,⁷ which showed increased survival times and decreased fungal burdens. Active immunization with a mixture of Hsp60 and an immunodominant *H. capsulatum* antigen increases the survival rates mediated by CD4⁺ lymphocytes, IFN- γ , IL-12, and IL-10, during the induction phase of vaccination.⁹ Furthermore, passive immunization with monoclonal antibodies directed against Hsp60 from *H. capsulatum* decreases the organ fungal burden, modify the course of experimental histoplasmosis, and increases the survival rates of C57BL/6 mice.¹⁹ The immunoprotective antifungal effect of Th1 response, with elevated IFN- γ and IL-2 levels, has also been observed after a lethal infection with *C. immitis* of mice immunized with a 63 kDa urease, the homologous of the *H. capsulatum* 60 kDa heat-shock protein, or DNA encoding the *C. immitis* urease.²³

A 43 kDa (Gp43) glycoprotein from the CW of *P. brasiliensis* is the main antigen used in a variety of diagnostic serological tests.⁸ Monoclonal antibodies directed against this glycoprotein reduce the fungal burden in the lung and spleen of mice infected with this fungus.⁴ The use of a DNA vaccine encoding an epitope of Gp43 (known as P10), before and during the infection with *P. brasiliensis* in mice, decreases the fungal load in the lung, spleen, and liver, and increases IFN- γ production.³⁷ Likewise, the use of antibodies directed against H2B from *H. capsulatum*, a cell surface histone-like protein, decreases the inflammation and fungal load in organs from C57BL/6 mice with experimental histoplasmosis, which results in higher survival rates, as compared with the control group.³² These protective effects might be associated with increased production of IFN- γ . Comparable results have been obtained using a fraction of the CW proteins (TX114-DF) from *Coccidioides posadasii* in C57BL/6 mice. This protein fraction contains a 43 kDa immunodominant antigen that induces T cell proliferation and the secretion of high levels of IFN- γ in *in vitro* assays, indicating a strong Th1 response.⁴⁷

Certain fungal antigens, such as the 120 kDa adhesin from *B. dermatitidis* (known as WI 1), elicit immunoprotective Th1 and Th2 responses. This protein increases the survival rates of infected C57BL/6 mice. Noteworthy, mice produce elevated titers of a mixture of antibodies such as IgG2a and IgG3, which are associated with cellular immunity, but also IgG1 and IgG2b, which are more closely related to humoral immune response.⁵¹ During *C. albicans* infections, the use of specific antibodies against β -1,2-mannotriose, a CW glycan, conjugated with peptide fractions of the fructose biphosphate aldolase, methylhydroxyglutamate-monomethyltransferase, and phosphoglycerate kinase, induces a protective response in infected C57BL/6 and BALB/c mice, measured by the increase of its survival rates.⁵²

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

This paper constitutes a partial fulfillment of the requirements of the Graduate Program in Biomedical Sciences of the UJED. CAAF thanks the scholarship No. 201509 granted by the Consejo Nacional de Ciencia y Tecnología (CONACyT), Mexico.

References

- Bernardes-Engemann AR, Costa RC, Miguens BR, Penha CV, Neves E, Pereira BA, et al. Development of an enzyme-linked immunosorbent assay for the serodiagnosis of several clinical forms of sporotrichosis. *Med Mycol*. 2005;43:487–93.
- Bernardes-Engemann AR, Penha CV, Benvenuto F, Braga JU, Barros ML, Costa RC, et al. A comparative serological study of the SsCBF antigenic fraction isolated from three *Sporothrix schenckii* strains. *Med Mycol*. 2009;47:874–8.
- Bromuro C, La Valle R, Sandini S, Urbani F, Ausiello CM, Morelli L, et al. A 70 kilodalton recombinant heat shock protein of *Candida albicans* is highly immunogenic and enhances systemic murine candidiasis. *Infect Immun*. 1998;66:2154–62.
- Buissá-Filho R, Puccia R, Marques AF, Pinto FA, Muñoz JE, Nosanchuk JD, et al. The monoclonal antibody against the mayor diagnostic antigen of *Paracoccidioides brasiliensis* mediates immune protection in infected BALB/c mice challenged intratracheally with fungus. *Infect Immun*. 2008;76:3321–8.
- Carlos IZ, Sgarbi DBG, Angluster J, Alviano CS, Silva CL. Detection of cellular immunity with the soluble antigen of the fungus *Sporothrix schenckii* in the systemic form of the disease. *Mycopathologia*. 1992;117:139–44.
- Cole GT, Krause D, Zhu ZW, Seshan KR, Wheat RW. Composition, serological reactivity, and immunolocalization of a 120-kilodalton tube precipitin antigen of *Coccidioides immitis*. *Infect Immun*. 1990;58:179–88.
- De Bastos AR, Gomez FJ, Almeida CM, Deepe GS. Vaccination with heat shock protein 60 induces a protective immune response against experimental *Paracoccidioides brasiliensis* pulmonary infection. *Infect Immun*. 2008;76:4214–21.
- De Camargo Z, Unterkircher C, Campoy SP, Travassos LR. Production of *Paracoccidioides brasiliensis* exoantigens for immunodiffusion tests. *J Clin Microbiol*. 1988;26:2147–51.
- Deepe GS, Gibbons RS. Cellular and molecular regulation of vaccination with heat shock protein 60 from *Histoplasma capsulatum*. *Infect Immun*. 2002;70:3759–67.
- De Mattos Grosso D, De Almeida SR, Mariano M, Lopes JD. Characterization of gp70 and anti-gp70 monoclonal antibodies in *Paracoccidioides brasiliensis* pathogenesis. *Infect Immun*. 2003;71:6534–42.
- De Meyer EM, De Beer ZW, Summerbell RC, Moharram AM, De Hoog GS, Vismer HF, et al. Taxonomy and phylogeny of new wood-and-soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras*–*Sporothrix schenckii* complex. *Mycologia*. 2008;100:647–61.
- Dickerson CL, Taylor RL, Drutz DJ. Susceptibility of congenitally athymic (nude) mice to sporotrichosis. *Infect Immun*. 1983;40:417–20.
- Fernandes GF, Dos Santos PO, Rodrigues AM, Sasaki AA, Burger E, De Camargo ZP. Characterization of virulence profile, protein secretion and immunogenicity of different *Sporothrix schenckii sensu stricto* isolates compared with *S. globosa* and *S. brasiliensis* species. *Virulence*. 2013;4:1–9.
- Fernandes GF, Lopes-Bezerra LM, Bernardes-Engemann AR, Schubach TM, Dias MA, Pereira SA, et al. Serodiagnosis of sporotrichosis infection in cats by enzyme-linked immunosorbent assay using specific antigen, SsCBF, and crude exoantigens. *Vet Microbiol*. 2011;147:445–9.
- Gomez FJ, Allenderoer R, Deepe GS. Vaccination with recombinant heat shock protein 60 from *Histoplasma capsulatum* protects mice against pulmonary histoplasmosis. *Infect Immun*. 1995;63:2587–95.
- Gomez FJ, Gomez AM, Deepe GS. Protective efficacy of a 62-kilodalton antigen, HIS 62, from the cell wall and cell membrane of *Histoplasma capsulatum* yeast cells. *Infect Immun*. 1991;59:4459–64.
- Gomez MJ, Torosantucci A, Arancia S, Maras B, Parisi L, Cassone A. Purification and biochemical characterization of a 65-kilodalton mannoprotein (MP65), a main target of anti-*Candida* cell-mediated immune responses in humans. *Infect Immun*. 1996;64:2577–84.
- Gorin PA, Haskins RH, Travassos LR, Mendonça-Prevato L. Further studies on the rhamnomannans and acidic rhamnomannans of *Sporothrix schenckii* and *Ceratocystis stenoceras*. *Carbohydr Res*. 1977;55:21–33.
- Guimaraes AJ, Frases S, Gomez FJ, Zancope-Oliveira RM, Nosanchuk JD. Monoclonal antibodies to heat shock protein 60 alter the pathogenesis of *Histoplasma capsulatum*. *Infect Immun*. 2009;77:1357–67.
- Hostetter MK. Adhesin and ligands involved in the interaction of *Candida* spp. with epithelial and endothelial surfaces. *Clin Microbiol Rev*. 1994;7:29–42.
- Klein BS, Hogan LH, Jones JM. Immunologic recognition of a 25-aminoacid repeat arrayed in tandem on a major antigen of *Blastomyces dermatitidis*. *J Clin Invest*. 1993;92:330–7.
- Klein BS, Jones JM. Isolation, purification, and radiolabeling of a novel 120-kDa surface protein on *Blastomyces dermatitidis* yeasts to detect antibodies in infected patients. *J Clin Invest*. 1990;85:152–61.
- Li K, Yu JJ, Hung CY, Lehmann PF, Cole GT. Recombinant urease and urease DNA of *Coccidioides immitis* elicit an immunoprotective response against coccidioidomycosis in mice. *Infect Immun*. 2001;69:2878–87.
- Lloyd KO, Bittan MA. Isolation and purification of a peptide-rhamnomannan from the yeast form of *Sporothrix schenckii*. Structural and immunochemical studies. *J Immunol*. 1971;107:663–71.
- López-Romero E, Reyes-Montes MR, Pérez-Torres A, Ruiz-Baca E, Villagómez-Castro JC, Mora-Montes HM, et al. *Sporothrix schenckii* complex and sporotrichosis, an emerging health problem. *Future Microbiol*. 2011;6:85–102.
- Maia DC, Sassá MF, Placeres MC, Carlos IZ. Influence of Th1/Th2 cytokines and nitric oxide in murine systemic infection induced by *Sporothrix schenckii*. *Mycopathologia*. 2006;161:11–9.
- Marimon R, Cano J, Gené J, Sutton DA, Kawasaki M, Guarro J. *Sporothrix brasiliensis*, *S. globosa*, and *S. mexicana*, three new *Sporothrix* species of clinical interest. *J Clin Microbiol*. 2007;45:3198–206.
- Marimon R, Gené J, Cano J, Guarro J. *Sporothrix luriei*: a rare fungus from clinical origin. *Med Mycol*. 2008;46:621–5.
- Nascimento RC, Almeida SR. Humoral immune response against soluble and fractionate antigens in experimental sporotrichosis. *FEMS Immunol Med Microbiol*. 2005;43:241–7.
- Nascimento RC, Espíndola NM, Castro RA, Teixeira PA, Penha CV, Lopes-Bezerra LM, et al. Passive immunization with monoclonal antibody against 70-kDa putative adhesin of *Sporothrix schenckii* induces protection in murine sporotrichosis. *Eur J Immunol*. 2008;38:3080–9.
- Nimrichter L, Rodrigues ML, Rodrigues EG, Travassos LR. The multitude of targets for the immune system and drug therapy in the fungal cell wall. *Microbes Infect*. 2005;7:789–98.
- Nosanchuk JD, Steenbergen JN, Shi L, Deepe GS, Casadevall A. Antibodies to a cell surface histone-like protein protect against *Histoplasma capsulatum*. *J Clin Invest*. 2003;112:1165–75.
- Penha CV, Lopes-Bezerra LM. Concanavalin-A-binding cell wall antigens of *Sporothrix schenckii*: a serological study. *Med Mycol*. 2000;38:1–7.
- Pontón J, Omaetxebarria MJ, Elgueza N, Alvarez M, Moragues MD. Immunoreactivity of the fungal cell wall. *Med Mycol*. 2001;39:101–10.
- Prevato JO, Gorin AJ, Haskins RH, Travassos LR. Soluble and insoluble glucans from different cell types of the human pathogen *Sporothrix schenckii*. *Exp Mycol*. 1979;3:92–105.
- Ramos SM, Vasconcelos C, Carneiro S, Cestari T. Sporotrichosis. *Clin Dermatol*. 2007;25:181–7.
- Rittner MG, Muñoz JE, Marques AF, Nosanchuk JD, Taborda CP, Travassos LR. Therapeutic DNA vaccine encoding peptide P10 against experimental paracoccidioidomycosis. *PLoS Neglected Tropical Diseases*. 2012;6(2):e1519.
- Rivitti EA, Aoki V. Deep fungal infections in tropical countries. *Clin Dermatol*. 1999;17:171–90.
- Rocha MM, Dassin T, Lira R, Lima EL, Severo LC, Londero AT. Sporotrichosis in patient with AIDS: report of a case and review. *Rev Iberoam Micol*. 2001;18:133–6.
- Ruiz-Baca E, Cuéllar-Cruz M, López-Romero E, Reyes-Montes MR, Toriello C. Fungal cell wall antigens for diagnosis of invasive fungal infections. In: *Fungal cell wall*. New York: Nova Science Publishers Inc.; 2013, in press.
- Ruiz-Baca E, Mora-Montes HM, López-Romero E, Toriello C, Mojica-Marín V, Urtiz-Estrada N. 2D-immunoblotting analysis of *Sporothrix schenckii* cell wall. *Mem Inst Oswaldo Cruz*. 2011;106:248–50.
- Ruiz-Baca E, Toriello C, Pérez-Torres A, Sabanero-López M, Villagómez-Castro JC, López-Romero E. 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*. 2009;47:185–96.
- Scott EN, Muchmore HG. Immunoblot analysis of antibody responses to *Sporothrix schenckii*. *J Clin Microbiol*. 1989;27:300–4.
- Shiraishi A, Nakagaki K, Arai T. Experimental sporotrichosis in congenital athymic (nude) mice. *J Reticuloendothelial Soc*. 1979;26:333–6.
- Tachibana T, Matsuyama T, Mitsuyama M. Characteristic infectivity of *Sporothrix schenckii* to mice depending on routes of infection and inherent fungal pathogenicity. *Med Mycol*. 1997;36:1–27.
- Tachibana T, Matsuyama T, Mitsuyama M. Involvement of CD4+ T cells and macrophages in acquired protection against infection with *Sporothrix schenckii* in mice. *Med Mycol*. 1999;37:397–404.
- Tarcha EJ, Basrur V, Hung CY, Gardner MJ, Cole GT. A recombinant aspartyl protease of *Coccidioides posadasii* induces protection against pulmonary coccidioidomycosis in mice. *Infect Immun*. 2006;74:516–27.
- Teixeira PA, De Castro RA, Nascimento RC, Tronchin G, Pérez-Torres A, Lazéra M, et al. Cell surface expression of adhesins for fibronectin correlates with virulence in *Sporothrix schenckii*. *Microbiology*. 2009;155:3730–8.
- Travassos LR, Gorin PA, Lloyd KO. Discrimination between *Sporothrix schenckii* and *Ceratocystis stenoceras* rhamnomannans by proton and carbón-13 magnetic resonance spectroscopy. *Infect Immun*. 1974;9:674–80.
- Travassos LR. *Sporothrix schenckii*. In: Szaniszlo PJ, editor. *Fungal dimorphism: with emphasis on fungi pathogenic for humans*. New York: Plenum Press; 1985. p. 121–63.
- Wüthrich M, Chang WL, Klein BS. Immunogenicity and protective efficacy of the WI-1 adhesin of *Blastomyces dermatitidis*. *Infect Immun*. 1998;66:5443–9.
- Xin H, Cutler JE. Vaccine and monoclonal antibody that enhance mouse resistance to candidiasis. *Clin Vaccine Immunol*. 2011;18:1656–67.