Rev Iberoam Micol. 2011;**28(1)**:1–25

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Review article

Experimental medical mycological research in Latin America - a 2000-2009 overview

Gioconda San-Blas[a,*], Eva Burger[b]

[a] *Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela*
[b] *Universidade de São Paulo, Brazil*

ARTICLE INFO

Article history:
Received 8 July 2010
Accepted 24 November 2010
Available online 15 December 2010

Keywords:
Latin America
Fungal pathogens
Mycosis
Diagnosis
Vaccines
Cell biology
Taxonomy
Immunology
Antifungals

ABSTRACT

An overview of current trends in Latin American Experimental Medical Mycological research since the beginning of the 21st century is done (search from January 2000 to December 2009). Using the PubMed and LILACS databases, the authors have chosen publications on medically important fungi which, according to our opinion, are the most relevant because of their novelty, interest, and international impact, based on research made entirely in the Latin American region or as part of collaborative efforts with laboratories elsewhere. In this way, the following areas are discussed: 1) molecular identification of fungal pathogens; 2) molecular and clinical epidemiology on fungal pathogens of prevalence in the region; 3) cell biology; 4) transcriptome, genome, molecular taxonomy and phylogeny; 5) immunology; 6) vaccines; 7) new and experimental antifungals.

Investigación en micología médica experimental en América Latina-perspectiva general entre 2000–2009

Palabras clave:
América Latina
Hongos patógenos
Micosis
Diagnóstico
Vacunas
Biología Celular
Taxonomía
Inmunología
Antifúngicos
Drogas Antifúngicas

RESUMEN

Se presenta una revisión de las más importantes líneas de investigación en micología médica experimental en América Latina desde el inicio del siglo XXI (búsqueda bibliográfica desde enero de 2000 a diciembre de 2009). Usando las bases de datos PubMed y LILACS, los autores hemos escogido publicaciones en hongos patógenos de importancia clínica que, de acuerdo a nuestra opinión, son las más relevantes por su novedad, interés e impacto internacional, basadas en investigaciones realizadas totalmente en la región latinoamericana o como parte de esfuerzos colaborativos con laboratorios de otras partes del mundo. De esta forma, discutimos las siguientes áreas: 1) identificación molecular de patógenos fúngicos; 2) epidemiología clínica y molecular de hongos patógenos prevalentes en la región; 3) biología celular; 4) transcriptoma, genoma, taxonomía y filogenia moleculares; 5) inmunología; 6) vacunas; 7) antifúngicos nuevos o experimentales.

Recent data[208] indicate that fungal diseases in Brazil do carry a high toll on fatal outcome of systemic mycoses. Although Prado et al's figures are limited to that country, it is reasonable to assume that given the similarities in regional and local health services and other social factors, their results may be representative of events in other Latin American countries. So, according to the authors, death tolls in Brazil within the period 2005-2006, amount to 44.6% (paracoccidioidomycosis, PCM), 26.8% (cryptococcosis), 16.3% (candidiasis), 5.6% (histoplasmosis), 5.0% (aspergillosis), 0.9% (zygomycosis), and 0.8% (coccidioidomycosis) of total patients treated for these diseases.[208] It is obvious, then, that Latin American countries suffer from a significant burden of systemic mycoses, which need to be addressed not only in terms of public health policies but also, and equally important, with an aggressive program on

* Corresponding author.
E-mail address: gsanblas@gmail.com (G. San-Blas).

1130-1406/$ – see front matter
doi:10.1016/j.riam.2010.11.004

academic, experimental and clinical research aimed to understand the phenomena underlying this serious and ever growing health problem.

This review highlights publications chosen by the authors as representative of the most relevant research in experimental medical mycology carried out in Latin America in the period January 2000-December 2009. Seven areas are covered and discussed within the framework of international research: 1) molecular identification of fungal pathogens; 2) molecular and clinical epidemiology on fungal pathogens of prevalence in the region; 3) cell biology; 4) transcriptome, genome, molecular taxonomy; 5) immunology; 6) vaccines; 7) new and experimental antifungals.

Searching in PubMed and the Latin American LILACS data bases (key words mycosis + <country> Jan 2000-December 2009), we found 1958 publications assigned to Latin American countries, either made entirely in the region or in collaboration with laboratories elsewhere, of which 1113 (57%) correspond to Brazil, 280 to Mexico, 229 to Argentina, 88 to Colombia, 86 to Venezuela, and 44 to Chile. *Paracoccidioides brasiliensis* and PCM comprise 565 (29%) of all regional publications in medical mycology, pointing to the impact of this mycosis and its causal agent in Latin America, only geographical region to host the fungus.[231]

Molecular identification of fungal pathogens

A major obstacle to the successful treatment of invasive fungal infections is the paucity of rapid, sensitive and specific methods that would help in the early diagnosis of fungal infections. PCR assays for diagnostic purposes are being extensively used though the method still lacks standardization and cannot be used as the sole test for early detection or for the purpose of defining invasive fungal infection [for a recent review, see 229].

In Latin America, PCR methodology for identification and diagnostic purposes has been applied ever since it appeared in the scientific literature as a promising technique. The *Histoplasma capsulatum* H or M antigens, pluripotent glycoproteins that elicit both humoral and T cell-mediated immune responses, are proteins whose genes have been used for the design of primers aimed at molecular diagnosis.[32,111] Bracca et al[32] developed a highly specific and sensitive semi-nested PCR assay in which three oligonucleotides, placed at the fifth exon of the gene encoding the H antigen, were chosen for their ability to differentiate *H. capsulatum* sequences from sequences of other fungal ß-glucosidases in the databases. Meanwhile, Guedes et al[111] used the M-antigen gene (highly homologous to catalases) to design four oligonucleotide sequences in the less homologous regions, for application in a one-step PCR detection and identification of *H. capsulatum* var. *capsulatum*. De Aguirre et al[72] used PCR technology in an enzyme immunoassay format for the rapid differentiation of *Aspergillus* species from other medically important opportunistic molds and yeasts. With oligonucleotide probes, directed to the ITS2 region of ribosomal DNA from several *Aspergilli*, they were able to differentiate 41 isolates; a single DNA probe to detect all seven of the most medically important *Aspergillus* species (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger, Aspergillus terreus*, *Aspergillus ustus*, and *Aspergillus versicolor*) was also designed.

In a prospective study in a high-risk population of candidemia, Moreira-Oliveira et al[171] used ITS5 and ITS4 primers for PCR, followed by sequencing of products for *Candida* spp. identification. They reported 72.1 and 91.2% sensitivity and specificity, respectively. A combination of PCR and RFLP helped in the identification of *Candida* species in immunocompromised and seriously diseased patients.[206] *Candida albicans* was the species most frequently observed, except for the group of newborns who were infected preferentially by *Candida parapsilosis*.[206] RAPD in combination with the OPE-18 primer was reported to be a very specific and sensitive method for the identification of *Candida glabrata*, *Candida guilliermondii*, *Candida tropicalis*, *Candida pelliculosa*, *C. albicans*, *Candida krusei*, and *Candida lusitaniae*.[14] RAPD was also used for the correct identification of *C. albicans* and *Candida dubliniensis*.[20] In 2006, the latter species was detected for the first time in Venezuela by means of a PCR approach.[34] A different methodological approach for the identification of candidemia by *C. albicans*, *C. tropicalis*, and *C. parapsilosis* was used by Berzaghi et al.[24] With the help of an inhibition enzyme-linked immunosorbent assay (ELISA) and anti-65-kDa monoclonal antibody, they were able to detect a 65-kDa antigen that presented three different patterns of antigenemia when tested against sera from patients: i) total clearance of antigenemia, ii) initial clearance and relapse of antigenemia, and iii) partial clearance of antigenemia. These results suggest that detection of the 65-kDa protein may be a valuable tool for the differential diagnosis of candidemia caused by either of these *Candida* species.

The antigen 2/proline-rich antigen has been used for the PCR detection of *Coccidioides posadasii*.[26,71] This antigen is common to *C. posadasii* and *Coccidioides immitis*.

Diagnostic primers in *P. brasiliensis* have been designed primarily from the nucleotide sequence of its reference antigen, gp43.[25,103] Some of the gp43 sequences were taken from regions where later studies[166] revealed the presence of informative and non informative nucleotide substitutions in this highly polymorphic gene,[166] a result that may influence the use of gp43 as a universal reference antigen.[231] A second set of diagnostic primers tested in clinical samples is that of San-Blas et al.[230] They were designed from two specific DNA fragments (Mw 0.72 and 0.83 Kb) common to and specific for all *P. brasiliensis* samples, generated when using the arbitrary primer OPG18 (Operon Biotechnology).[37] Such primers were capable of rapidly identifying *P. brasiliensis* DNA from sputum and cerebrospinal fluid of PCM patients.[230]

A duplex polymerase chain reaction (PCR) targeting the ITS1-5.8S-ITS2 region of the ribosomal DNA was designed for rapid and specific identification of 69 *Fonsecaea pedrosoi* isolates; 4 *Fonsecaea compacta* samples and several other dematiaceous isolates did not produced identification bands.[73] The frequency of *Fonsecaea*-positive results was similar between duplex PCR (68.0%) and morphology (67.0%). However, 4% isolates were positive by duplex PCR but negative by morphology, indicating that PCR method may be the test of choice when dealing with samples unable to produce conidia. On the other hand, 3% samples were positive by morphology and negative by *Fonsecaea*-specific PCR. These isolates have high similarity to the genus *Phialophora* when DNA sequencing analyses were performed.

Epidemiology of prevalent fungal pathogens in the region

Clinical epidemiology

Antimicrobial resistance surveillance serves for the detection and tracking of resistance trends and emerging new resistance threats, and also as a means to monitor the prevalent pathogens causing serious infections. In order to address effectively any of these objectives, the availability of a geographically diverse collection of isolates from clinically important sites of infection is essential.[203] Very few programs provide information on fungal infections and antifungal resistance, among them, the ARTEMIS Global Antifungal Susceptibility Program (ARTEMIS Program) and the Regional Laboratory Network for Surveillance of Invasive Fungal Infections and Antifungal Susceptibility in Latin America, both mainly focused on candidemia from several *Candida* spp. Of recent formation and consequently, few reports to date, the Regional

Table 1
Species distribution (%) of *Candida* bloodstream isolates in different Latin American regions.

Geographical region/city/country	*C. albicans*	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	Reference
Brazil, 9 cities	40.9	20.5	20.9	4.9	60
Caracas, Venezuela	46.7	6	19	9.2	78
Chile	47	21	14	3	236
BuenosAires, Argentina	41	29	16	3	214
Monterrey, Mexico	31.9	37.9	14.8	8.0	109

Network is coordinated by the Essential Medicines, Vaccines, and Health Technologies Unit of the Pan American Health Organization, with the technical and financial support of the National Center for Microbiology of the Carlos III Health Institute (Spain), and the technical support of the Microbiology Department of the Dr. C. Malbrán National Institute on Infectious Diseases (Argentina) and the Microbiology Unit of the Parasitology Service of the Adolfo Lutz Institute (Brazil).[67] The Network's main objectives are epidemiological surveillance of invasive fungal infections through detection of antifungal resistance and identification of emergent, invasive fungal infections; establishment of norms and common protocols for early diagnosis of mycoses; strengthening coordination, communications, and transference mechanisms among participant countries.[60] The older ARTEMIS program was initiated in 2001 to provide focused surveillance of the activities of fluconazole and voriconazole against *Candida* spp. causing invasive infections, and to provide continuous development and validation of various broth- and agar-based antifungal susceptibility test systems. The ARTEMIS Program has provided a massive amount of data; it uses a central reference laboratory and an international network of 105 participating centers as sources of clinical isolates[203]; several Latin American laboratories provide essential information within this program. Their results indicate that more than 90% of invasive infections due to *Candida* spp. are attributed to five species, *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*. Although *C. albicans* remains the dominant species causing bloodstream infections (BSI), the frequency of occurrence varies throughout the world from 37% in Latin America to 70% in Norway. Most notable is the very low frequency of *C. glabrata* as a cause of BSI in Latin America, where only 4% to 7% of *Candida* BSIs are attributed to this species.[104,204] The frequency of isolation of *C. glabrata* from blood cultures follows a trend toward decreased frequency in Latin America (7.4% to 4.7% of BSIs), Europe (10.5% to 8.8%), and the Asia-Pacific region (12.1% to 7.2%) between 2002 and 2004.[171] The frequency of invasive candidiasis due to *C. parapsilosis* has increased in recent years, most notably in Latin America. Whereas *C. tropicalis* is only the fourth most common species of *Candida* causing BSI in North America (7% of BSIs), it ranks second in Latin America (20%) and is more common than *C. glabrata* in the Asia-Pacific region (14-21% vs. 10-12%, respectively). *C. guilliermondii* and *Candida rugosa* are relatively uncommon species of *Candida* that appear to be increasing in frequency as agents of invasive candidiasis. These two species can be found in Latin America, where they each account for 3% to 5% of all candidemias and may be more common than either *C. glabrata* or *C. krusei*.[60,78,104,109]

The above mentioned global figures hide the documented geographic differences in rates and epidemiology of candidemia in different countries and cities within them (Table 1). Prospective candidemia surveillance in 11 medical centers located in 9 major Brazilian cities (March 2003 to December 2004) indicated that *C. albicans* was the most common species (40.9%), followed by *C. tropicalis* (20.9%) and *C. parapsilosis* (20.5%).[60] Further candidaemia surveillance studies in Brazil[104] indicated that in the genetically heterogeneous taxon *C. parapsilosis* (*C. parapsilosis*, *Candida orthopsilosis* and *Candida metapsilosis*), the prevalence of each species among 141 bloodstream isolates was 88%, 9% and 3%, respectively. All isolates but three 5-fluorocytosine-resistant *C. orthopsilosis* were susceptible to polyenes, triazoles and caspofungin.

Similar studies[78] in six health care centers of Caracas, Venezuela, from January 2003 through August 2005, indicated that *C. albicans* was the most frequently isolated yeast (46.7%), followed by *C. tropicalis* (19.0%), *C. glabrata* (9.2%) and *C. parapsilosis* (6.0%). During the first year of an ongoing surveillance program of invasive fungal infections in 13 hospitals in Chile, Silva et al[236] found that *C. albicans* (40.8%), *C. parapsilosis* (13.1%), *C. tropicalis* (10%) and *Cryptococus neoformans* (10%) were the most common yeast species. A multicenter study performed to determine the species distribution associated to candidemias in Argentina[214] also brought about similar results, that is, *C. albicans* (40.75%), *C. parapsilosis* (28.67%), *C. tropicalis* (15.84%), *Candida famata* (3.77%), *C. neoformans* (3.77%), *C. glabrata* (2.64%), and others (4.53%). Interestingly, *C. parapsilosis* (37.9%) was the most frequent species found by González et al[109] in a 3-year surveillance program (2004 to 2007) in Monterrey, Mexico, followed by *C. albicans* (31.9%), *C. tropicalis* (14.8%), *C. glabrata* (8.0%), among the most frequent. The species distribution differed with the age of the patients, a result also documented by Pfaller and Diekema.[203]

C. dubliniensis is associated with oral candidiasis in immunodepressed individuals. Using classical phenotypic methods combined with PCR techniques, Jewtuchowicz et al[114] found that this species is present in 4.4% of periodontal pockets of immunodepressed Argentinean patients. *C. albicans* was the most frequent species, corresponding to 24.4% (44/180). Other non-*C. albicans* species were found, among them *C. parapsilosis*, *C. tropicalis*, and *C. guilliermondii*.

These studies were always accompanied by screening of resistance to antifungals such as azoles and echinocandins, indicating a wide range of variability in the susceptibility of strains. Together, these data point to the importance of local and regional surveillance studies to guide physicians towards the most effective treatment of candidiasis and other fungal diseases.

Retrospective studies characterizing acute/subacute PCM incidence in the Botucatu area, São Paulo State, Brazil, from 1969 to 1999 and their relationship with climate variables (antecedent precipitation, air temperature, soil water storage, absolute and relative air humidity, and Southern Oscillation Index) have been done by Barrozo et al.[17] They concluded that correlations may reflect enhanced fungal growth after increase in soil water storage in the longer term and greater spore release with increase in absolute air humidity in the short term.

Molecular epidemiology

The basidiomycetous yeasts *C. neoformans* and *Cryptococcus gattii* are closely related sibling species that cause respiratory and neurological disease in humans and animals. Within these two recognized species, phylogenetic analysis reveals cryptic species or molecular types within the pathogenic *Cryptococcus* species complex, corresponding to serotypes A (*C. neoformans* var. *grubii*; VNI, VNII), D (*C. neoformans* var. *neoformans*; VNIV), AD (Hybrid; VNIII), B and C (*C. gattii*; VGI-VGIV).[92,119,163] To acquire basic knowledge of

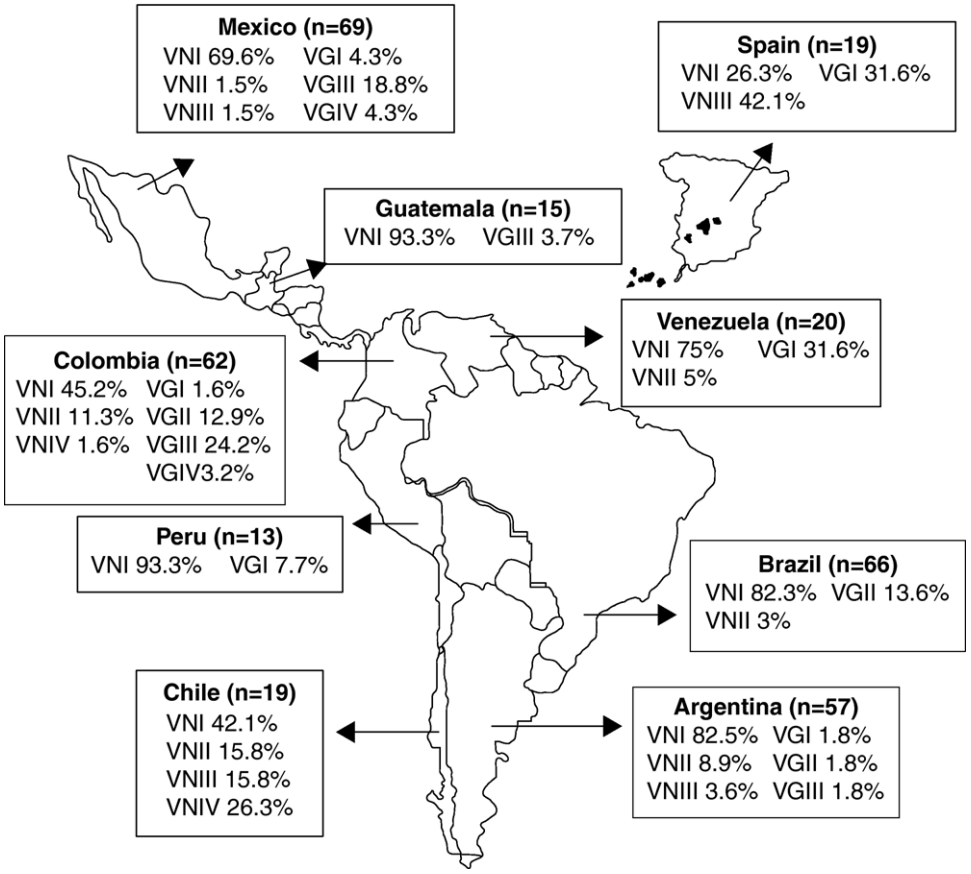

Figure 1. Geographic distribution of the molecular types obtained from IberoAmerican *Cryptococcus neoformans* isolates by polymerase chain reaction fingerprinting and URA5 gene restriction fragment length polymorphis analysis (total numbers studied per country given in parentheses).[163] Reproduced by permission.

C. neoformans in IberoAmerican countries, 266 clinical, 7 veterinary, and 67 environmental isolates from Argentina, Brazil, Chile, Colombia, Mexico, Peru, Venezuela, Guatemala, and Spain were typed by means of the M13 polymerase chain reaction-fingerprinting and orotidine monophosphate pyrophosphorylase (URA5) gene restriction fragment length polymorphism (RFLP) analysis with HhaI and Sau96I in a double digest.[163] The majority of the isolates (68.2%) were VNI (*C. neoformans* var. *grubii*, serotype A), which agrees with the fact of this variety being the cause of most human cryptococcal infections worldwide, particularly in HIV-positive patients. Of the remaining, 5.6% were VNII; 4.1% VNIII; 1.8%, VNIV; 3.5% VGI; 6.2% VGII; 9.1% VGIII, and 1.5% VGIV. Chile and Spain shared similar molecular types, with a large number (15.8% and 42.1%, respectively) of molecular type VNIII isolates (AD hybrids). VNIV serotype D isolates were present only in Chile (26.3%). Patients with no known risk factors had *C. gattii* (VGI-VGIV) as the main fungal agent (fig. 1).[163]

Similar studies in Colombia[84] indicated a prevalence of serotype A (91.1%) followed by serotypes B (8.4%) and C (0.5%) in clinical samples, figures that moved to 44.2, 42.6 and 13.2%, respectively, in environmental isolates. No serotype D or AD samples were isolated. With the same technique used by Meyer et al,[163] the majority of clinical serotype A and environmental serotype B isolates were grouped into the molecular types VNI (98.1%) and VGII (100%), respectively. Molecular type VGII was the predominant genotype (77.7%) in both clinical and environmental Colombian *C. gattii* isolates. This contrasts with previous reports in which VGII was only found occasionally in tropical and subtropical regions.[163]

The most common molecular type found in Brazil was VNI (64%), followed by VGII (21%), VNII (5%), VGIII (4%), VGI and VNIV (3% each), and VNIII (< 1%).[270] Primary cryptococcosis caused by *C. gattii*, molecular type VGII, prevailed in immunocompetent hosts, mainly young people and children, in the North and Northeast regions where *C. gattii* is endemic. On the other hand, in the Brazilian Southern region, sporadic infections by *C. gattii* were recorded. Overall, the most common molecular types were VNI (64%) and VGII (21%), followed by VNII (5%), VGIII (4%), VGI and VNIV (3% each), and VNIII (< 1%). Molecular type VGIV was not identified among the Brazilian isolates.[270]

Out of 72 Mexican clinical isolates (PCR-fingerprinting with the primer M13), 55 VNI, five VNII, three VNIII, one VNIV, two VGI, two VGII, two VGIII and two VGIV isolates were reported.[191] The results show that most cryptococcosis cases in Mexico are AIDS-related and are caused by *C. neoformans* var. *grubii*, genotypes VNI and VNII. In addition, this study revealed for the first time the presence of genotypes VNIV and VGII among Mexican clinical isolates. Hence, all genotypes that have been described for the *Cryptococcus* species complex are found in Mexico, indicating a much wider geographic distribution of genotypes than previously reported.

Using a different molecular approach, Díaz et al[77] employed sequence analysis of the intergenic spacer regions, IGSI and IGSII, the most rapidly evolving regions of the rDNA families. The IGSI region displays the higher genetic variability, represented by nucleotide base substitutions and the presence of long insertions/deletions (indels). In contrast, the IGSII region exhibits less heterogeneity and less extensive indels than the IGSI region. Both intergenic spacers contain short, interspersed repeat motifs, which can be related to length polymorphisms observed between sequences. Phylogenetic analyses, undertaken in the IGSI, IGSII and IGSI +5S rRNA + IGSII regions, revealed the presence of six major

phylogenetic lineages, some of which segregated into subgroups. The major lineages are represented by genotypes 1 (*C. neoformans* var. *grubii*), genotype 2 (*C. neoformans* var. *neoformans*), and genotypes 3 to 6 represented by *C. gattii*, not always coincident with the molecular types found in the previously reviewed data.[72,163,270]

H. capsulatum is a dimorphic fungus that has been recognized as an important worldwide pathogen, agent of histoplasmosis. The disease, which in some Latin American regions is a public health threat, presents a wide diversity of clinical manifestations. Studies on chromosomal band profiles of clinical isolates might shed light on the role of fungal genetic diversity in the evolution of different clinical forms of the disease. Using pulsed-field gel electrophoresis, Canteros et al[42] analyzed intact chromosomes of 19 clinical isolates of *H. capsulatum* isolated in Argentina, Mexico and Guatemala and the laboratory reference strain G186B from Panama. Chromosomal banding patterns, grouped in 13 different electrokaryotypes, ranged between 5 and 7 bands, 1.3 to 10 Mbp in size. Strain G186B produced five bands of approximately 1.1, 2.8, 3.3, 5.4 and 9.7 Mbp. Such chromosomal variability did not correlate with geographical or clinical source. In spite of the apparently high chromosome-length polymorphism, three clusters of identical patterns were identified. The largest group, karyotype I, included only Argentinean isolates of clinical origin, although this was not the only karyotype in harbouring isolates from this country. All Mexican *H. capsulatum* isolates were polymorphic among them.

Clinical observations in some Latin American countries indicate that the lymphocutaneous form of sporothricosis is prevalent in Mexico and Guatemala, whereas the fixed cutaneous form prevails in Colombia. Mesa-Arango et al[162] aimed to determine the genotypic and phenotypic relatedness among *Sporothrix schenckii* isolates in these countries. Clinical and environmental isolates of *S. schenckii* were subjected to RAPD analysis-PCR with 10-mer primers OPBG-01, OPBG-14, and OPBG-19. The 44 *S. schenckii* isolates fell into four major groups by hierarchical cluster analysis. Group I cluster together 25 out of 27 Mexican isolates, into two subgroups, Ia with 10 environmental isolates and Ib with 14 clinical isolates. Group II also split into two subgroups: IIa, Colombian isolates, and IIb, Guatemalan isolates. Groups III and IV each had only one clinical Mexican isolate. The low thermotolerance at 35 and 37 °C of the Colombian isolates could be associated with superficial skin lesions in patients with fixed clinical forms of sporotrichosis, the most frequent form of the disease in Colombia. Even though the isolates were grouped by geographical origins, a high degree of genotypic variability was observed among the isolates. Reporting a sporothrichosis epidemic in Rio de Janeiro, Brazil, Reis et al[210] demonstrated its zoonotic character using molecular methodology. For this, the RAPD technique with three different primers and DNA fingerprinting using the minisatellite derived from the wild-type phage M13 core-sequence allowed the authors to cluster 19 human and 25 cat *S. schenckii* isolates into 5-10 genotypes. The RAPD profiles of epidemic *S. schenckii* isolates could be distinguished from that of the United States-reference isolate, displaying 20% similarity to each primer and 60% when amplified with the M13 primer. DNA fingerprinting of *S. schenckii* isolated from the nails (42.8%) and the oral cavities (66%) of cats were identical to related human samples, suggesting that a common infection source for animals and humans in this epidemic, cats serving as a vehicle for dissemination of *S. schenckii*.

By cladistic analysis of partial sequences of the calmodulin gene using the maximum parsimony and neighbor-joining methods, Madrid et al[133] determined that one out of 25 isolates from Mexico (4%), one out of three isolates from Guatemala (33.3%), and two out of four isolates from Colombia (50%) belonged to *Sporothrix globosa*, while all other isolates belonged to *S. schenckii sensu stricto*, this being the first record of *S. globosa* from Mexico, Central and South America.

Cell biology

Metabolic and regulatory processes in growth and morphogenesis

Cell cycle and interaction between DNA replication, nuclei segregation and budding in *P. brasiliensis* have been poorly studied. Almeida et al[3] focused on the characteristics of the cell cycle profile of *P. brasiliensis* yeast cells during batch culturing and under the effects of benomyl, an antifungal drug known to promote a cell cycle arrest in the G2/M phases of *Saccharomyces cerevisiae*. Their results suggested that even though benomyl progressively blocks nuclear division of *P. brasiliensis* yeast form, treated cells retained their capacity for DNA replication.

Cells possess rapidly responding, highly complex signaling pathways to allow them to quickly adapt to a changing environment. Most prominent among them are the mitogen-activated protein kinase cascades. Some aspects of such complex systems are under study in Latin America. The cAMP-dependent protein kinase (PKA) from *C. albicans* is a tetramer composed of two catalytic subunits and two type II regulatory subunits encoded by *TPK1* and *TPK2*, respectively, whose autophosphorylation site in Ser180 possibly conforms a modulatory mechanism for *C. albicans* PKA activity in vivo.[289,290] *TPK1* is a positive regulator of the morphogenetic transition of *C. albicans* in the absence of the *TPK2* gene.[58] The loss of one catalytic isoform is not compensated by overexpression of the other.[247] During Y-M transition, a sharp increase in *TPK1* mRNA levels and in PKA-specific activity correlated with the onset of germ-tube formation in strain *tpk2Δ*, reinforcing the idea that Tpk1p is important for faster germ-tube appearance.

Bcy1p is a regulatory subunit of the PKA catalytic subunits TPK1 and TPK2.[100] *BCY1*$^{-}$ *C. albicans* yeast cells were used to generate a double *bcy1 tpk2* mutant,[47] with which it was proven that its constitutive PKA activity was cAMP independent, indicating that the cells harbored an unregulated phosphotransferase activity. Strains with one *BCY1* allele displayed pseudohyphae and true hyphae, while hyphal morphology was almost exclusive in strains having both *BCY1* alleles, suggesting a tight regulation of PKA activity for hyphal growth.[100] Further work[101] with mutants having heterozygous or homozygous deletions of TPK1 and/or TPK2 indicated that tpk1Δ/tpk1Δ strains developed a lower tolerance to saline exposure, heat shock and oxidative stress as well as defects in glycogen storage, whereas wild-type and tpk2Δ/tpk2Δ mutants were resistant to these stresses and accumulated higher levels of the polysaccharide, indicating that both isoforms play different roles in the stress response pathway and carbohydrate metabolism. In *Yarrowia lipolytica*, instead, an active PKA pathway promotes yeast-like growth and opposes mycelial development.[52]

The Y-M transition in *S. schenckii* responds to protein kinase C (PKC) effectors, indicating the involvement of PKC in this regulation. The presence of two pkc genes, *pkcSs-1* and *pkcSs-2*, were confirmed by Southern blot.[8] The latter has an ORF of 3942 nucleotides interrupted by five introns, to encode a protein of 1194 amino acids and 132.84 kDa. *pkcSs-2* is expressed at all intervals during the Y-M transition.[8] Also in *S. schenckii*, a $G_{\alpha i}$ subunit was found in a study aimed to search the role of G proteins in signal transduction, the first time such subunit was reported in a pathogenic fungus.[76] The cDNA sequence revealed a 1059 bp ORF encoding a 353 amino acid $G_{\alpha i}$ subunit of 41 kDa.

A homolog of the Pho85 cyclin-dependent kinase (Cdk) was found in *S. schenckii*.[75] Pho85 has been identified as a regulator of phosphate metabolism and modulator of the transcriptional response to nutritional signals. The *phoSs* gene consists of 990 bp, contains one intron, and encodes a protein of 306 amino acids. Expression of the *phoSs* gene decreased 30-fold during the Y-M transition. The addition of extracellular calcium accelerated the dimorphic transition and restored *phoSs* expression, suggesting

that PhoSs may participate in the control of the Y-M transition in *S. schenckii*.[75] As an initial step to understand the *PHO* pathway in *A. fumigatus*, de Gouvea et al.[74] characterized the PHO80 homologue, PhoB^{PHO80} and showed that the *ΔphoB*^{PHO80} mutant has a delayed germ tube emergence; by phenotypic and phosphate uptake analyses, the authors were able to establish a link between PhoB^{PHO80}, calcineurin and calcium metabolism. Several genes of the Pho complex, namely, *phoD*^{PHO84}, *phoE*^{PHO89}, *phoC*^{PHO81}, and vacuolar transporter Vtc4 were more expressed both in the *ΔphoB*^{PHO80} mutant background and under phosphate-limiting conditions of 0.1 mM P_i. *ΔphoB*^{PHO80} and *ΔphoD*^{PHO84} mutant strains were fully virulent in a murine low dose model for invasive aspergillosis.

The glyoxylate cycle, apparently involved in fungal pathogenicity (for a review, see Dunn et al[83]), allows for the use of lipids in the synthesis of glucose via acetate → citrate → isocitrate. Its two initial steps are identical to those in the citric acid cycle. After cleavage into succinate and glyoxylate and further condensation with acetyl-CoA, malate is produced. Malate synthase is present in *P. brasiliensis*; with a calculated 539 amino acids and a molecular mass of 60 kDa, the gene that encodes it (*Pbmls*) has 1617 bp.[287] The enzyme is located on the fungal cell surface and possibly plays a role in the binding of fungal cells to the host, behaving as an anchorless adhesion system.[178]

During the infective process, pathogenic fungi are subjected to a significant environmental stress, including exposure to reactive oxygen and nitrogen species produced by host cells. Mitochondria are the main source of reactive oxygen species which need to be controlled by detoxification mechanisms. Tudella et al[271] analyzed an alternative oxidase and an uncoupling protein in the respiratory chain of *A. fumigatus*. A functional respiratory chain (complex I-V) was demonstrated: adenosine 5'-diphosphate (ADP) induced an oligomycin-sensitive transition from resting to phosphorylating respiration, in the presence of the oxidizable substrates malate, glutamate, alpha-ketoglutarate, pyruvate, dihydroorotate, succinate, N,N,N',N'-tetramethyl-p-phenylenediamine and exogenous NADH. They were also able to demonstrate the presence of an alternative NADH-ubiquinone oxidoreductase, an alternative oxidase and an uncoupling protein in the respiratory chain of *A. fumigatus*. Cloning and functional expression of the mitochondrial alternative oxidase of *A. fumigatus* indicated that its gene (*Afaox*) is 1173 bp long, and encodes a 40 kDa protein.[135] In *P. brasiliensis* mitochondria, a complete (Complex I-V) functional respiratory chain was also demonstrated.[146] An alternative NADH-ubiquinone oxidoreductase, malate/NAD(+)-supported respiration, and alternative oxidase mechanism in the yeast form of the fungus suggested the existence of alternative respiratory chain pathways in addition to Complex I in *P. brasiliensis*.[146] Similar results were found in *C. albicans* mitochondria[51,226] and *C. parapsilosis*.[164] Because such pathways are absent in animal cells, they may be exceptional targets for the design of new chemotherapeutic agents. Other mitochondrial genes coding for enzymes involved in the respiratory electron-transport chain, namely, proline oxidase, riboflavin kinase, and cytochrome c oxidase, have been reported in the dermatophyte *Trichophyton rubrum*.[235]

Flavoprotein monooxygenases constitute a family of enzymes involved in a remarkably wide variety of oxidative reactions and are, therefore, oxidoreductases; they are mainly related to antioxidative stress in fungi and participate in several metabolic pathways. One such protein has been identified in *P. brasiliensis*.[140] It is the glycoprotein gp70, a concanavalin A-binding component recognized by about 96% of sera from untreated patients with PCM. Its gene encodes for a 79 kDa protein 718 aminoacids long. An increased PbGP70 transcript accumulation is observed under H_2O_2-induced oxidative stress, during fungal growth, and in macrophage phagocyted/bound yeasts. In this way, GP70 may work as a protector against oxidative stress and as elicitor of an immune response.[140] Also related to oxidative stress are catalases, whose main function is to prevent the oxidative damage triggered by the reactive oxygen species of the host. Three catalases have been reported in *P. brasiliensis*, two of which (CatA and PbCatC) are monofunctional catalases and the third one (CatP), a catalase peroxidase[54]; additionally, *P. brasiliensis* has both cytosolic and peroxisomal catalase isoenzymes and a single cytochrome-c peroxidase.[70] PbCatA manifested higher activity in the mycelial phase, during M-Y transition or endogenous oxidative stress. PbCatP showed higher activity in yeast cells since it is putatively involved in the control of exogenous reactive oxygen species. In *C. glabrata*, a high resistance to oxidative stress is mediated by a single catalase, Cta1p, controlled by the transcription factors Yap1p, Skn7p, Msn2p, and Msn4p.[66] *C. dubliniensis*, on the other hand, mounts an adaptive response to stress that leads to an increased survival against lethal doses of H_2O_2–like oxidants,[268] characterized by the induction of enzymes with known antioxidant function (glucose-6-phosphate dehydrogenase, superoxide dismutase and catalase). *C. dubliniensis* is less resistant to oxidants than *C. albicans*, displaying higher susceptibility to their toxic effects.[268]

Enzymes whose encoding genes are differentially expressed through the morphogenetic process have been reported. Ornithine decarboxylase (ODC) is associated to the metabolism of polyamines.[212] Early work indicated that in *C. albicans*, *Mucor rouxii*, and *Y. lipolytica*, the activity of ODC was higher in the mycelial phase; in *P. brasiliensis*, instead, it is the yeast phase that shows a higher activity of the enzyme, either at the extreme phases or through mycelial to yeast transition.[232] However, *PbrODC* expression remained constant at all stages of the fungal growth, a result that suggests a post-transcriptional regulation of the *PbrODC* product.[184] *C. immitis* ODC, cloned in the pETC*i*ODC plasmid under control of T7lac promoter, was produced in transformant strains *Escherichia coli* BL21(*DE3*), BL21(*DE3*)pLysS and BLR(*DE3*) (λ *DE3* lysogen), and EWH319 (*odc*- null mutant).[196] *E. coli* BL21(*DE3*)pLysS-pETC*i*ODC expressed the highest specific ODC activity, suggesting that this strain could be successfully used for protein structure and drug testing studies.

Calcineurin is a Ca^{2+}/calmodulin-dependent, serine/threonine-specific phosphatase essential for adaptation to environmental stress, growth, morphogenesis, and pathogenesis in many fungal species. Calcineurin controls hyphal and yeast morphology, M-Y dimorphism, growth, and Ca^{2+} homeostasis in *P. brasiliensis*.[40] In fungi calcineurin acts largely through regulating Crz1p-like transcription factors. The *A. fumigatus CRZ1* homologue *CrzA* was characterized[242]; it is involved in mediation of cellular tolerance to increased concentrations of calcium and manganese, also affecting conidiation. Additionally, *crzA*$^-$ mutants suffer altered expression of calcium transporter mRNAs under high concentrations of calcium, and loss of virulence when compared with the corresponding complemented and wild-type strains. The actual calcineurin A gene (*calA*), coding for the catalytic subunit, is involved in hyphal morphology related to apical extension and branching growth, as shown by the defective and drastically decreased filamentation in *calA*$^-$ *A. fumigatus* mutants.[136] Such mutants also showed an increased alternative oxidase (*aoxA*) mRNA accumulation and activity. The authors also identified four transcription factors (*zfpA*, *htfA*, *nosA*, and *ctfA*) that have increased mRNA expression in the absence of calcineurin, suggesting a negative regulation by this phosphatase. The deletion of the genes encoding these transcription factors yielded disturbed mRNA accumulation of *pmcA* and *pmcB* encoding calcium transporters. These deletion strains were also less susceptible to itraconazole, caspofungin, and SDS.

TOR (target of rapamycin) is a pathway by which a regulation is exerted on the translation of ribosomal proteins and, in yeast, of ribosome biogenesis. In *C. albicans*, morphogenesis towards hyphal

development is impaired by the addition of rapamycin, an inhibitor of TOR, in the culture medium. Additionally, lithium suppressed hyphal outgrowth in *C. albicans* in a way that also suggested inhibition of the TOR pathway.[145,146]

Proteinases occur naturally in all organisms, and are involved in a multitude of physiological reactions. They are divided into four major groups: serine-, cysteine (thiol)-, aspartic-, and metalloproteinases. Aspartyl- and serine-proteinases have been reported in several fungal species by Latin American research groups. Of the former, a 66 kDa, N-glycosylated secreted aspartyl protease (PbSAP) of *P. brasiliensis* was identified in the yeast cell wall. The expression of putative genes *CdSAP1*, *CdSAP2*, *CdSAP3*, and *CdSAP4* coding for secreted aspartyl proteases of *C. dubliniensis* were reported.[126,198] In addition, *CdSAP7*, *8*, *9*, and *10*, orthologous genes of *C. albicans*, were recognized in *C. dubliniensis* genome. The expression of *CdSAP1* and *2* was independent of the morphological stage of *C. dubliniensis*.[198] *CdSAP3* expression, instead, was related to the infective process of keratinocytes. Expression of *CdSAP4* predominated during the mycelial phase and the initial stage of keratinocyte infection. These results suggest a role of *C. dubliniensis* Saps as virulence factors, similar to those from *C. albicans*.[126] Genome mining and phylogenetic analyses revealed the presence of new members of the Sap superfamily in *C. tropicalis* (8), *C. guilliermondii* (8), *C. parapsilosis* (11) and *Candida lusitaniae* (3).[198] An extracellular aspartyl-related proteolytic activity was also detected in mycelial and conidial forms of *F. pedrosoi*. Pepstatin A was able to inhibit the growth of conidium and its transformation into mycelium, suggesting a possible participation of aspartyl peptidases in growth and differentiation.[193,194]

Exocellular serine-proteinases have been reported in *P. brasiliensis*[286] and *C. immitis*.[127] In the latter, the mycelial 25 kDa peptidase was able to degrade keratin while an additional 18 kDa serine peptidase activity was evidenced solely when casein was used as the substrate. In *P. brasiliensis*, Venancio et al[276] reported a kexin-like gene (*Pbkex2*) codifying for a kexin protein that belongs to the subtilase family of serine-proteinases. It is conformed by an open reading frame (ORF) of 2622 bp interrupted by one single 93 bp intron. The deduced protein sequence consists of 842 amino acid residues.[266,276] Also serine proteinases are the Lon proteins, with roles in the maintenance of mitochondrial DNA integrity and mitochondrial homeostasis. A *LON* gene homologue from *P. brasiliensis* (*PbLON*) was identified by Barros and Puccia.[16] *PbLON* ORF is within a 3,369-bp fragment interrupted by two introns located in the 3' segment; an *MDJ1*-like gene was partially sequenced in the opposite direction, sharing with *PbLON* a common 5' untranslated region.[19] The authors propose that this chromosomal organization might be functionally relevant, since Mdj1p is a type I DnaJ molecule located in the yeast mitochondrial matrix and is essential for substrate degradation by Lon and other stress-inducible ATP-dependent proteinases. An exocellular serine-thiol proteinase (PbST) activity was reported by Matsuo et al[147,148] in the yeast phase of *P. brasiliensis*. It was capable of cleaving proteins associated with the basal membrane, such as human laminin and fibronectin, type IV collagen and proteoglycans.[147,148] A 50-kDa serine peptidase was identified in *C. albicans* that was active over a broad pH range (5.0-7.2) and was able to hydrolyze some soluble human serum proteins and extracellular matrix components.[79] Conversely, when this isolate was grown in yeast carbon base supplemented with bovine serum albumin, a secretory aspartyl peptidase activity was measured, instead of metallo- and serine peptidases, suggesting that distinct medium composition induces different expression of released peptidases in *C. albicans*. Also in *C. albicans*, the *STE13ca* gene encodes for a dipeptidyl aminopeptidase A involved in the maturation of α-factor mating pheromone. This 2793 pb gene is homozygotic and encodes for a predicted protein of 930 amino acids with a molecular weight of 107 kDa. *STE13ca* increases its levels of expression in conditions of nutritional stress (proline as nitrogen source) and during formation of the germinal tube.[21]

Selective degradation of intracellular proteins in eukaryotic cells is carried out by a 26S proteasome/polyubiquitin system, in which polyubiquitin-labeled proteins are marked for destruction by the proteasome (for a review, see Sorais et al[240]). The basic unit of the 26S proteasome is the 20S proteasome, which in *C. albicans* yeast cells has a MW 640 kDa, distributed within 14 polypeptides.[88] The enzyme shows chymotrypsin-like, trypsin-like, and peptidylglutamyl-peptide-hydrolyzing activities. The regulation of its activity may be mediated, in part, by phosphorylation, as suggested by experiments in vivo, using homologous protein kinase CK2 as the substrate.[89]

Ecto-ATPases have been reported in *F. pedrosoi*[59] and *C. neoformans*.[116] In the presence of 1 mM EDTA, *F. pedrosoi* fungal cells hydrolyzed adenosine-5'-triphosphate (ATP) at a rate of 84.6 +/- 11.3 nmol Pi/ h/ mg mycelial dry weight, while a value of 29.36+/-3.36nmol Pi/h/10^8 cells was reported for *C. neoformans.* $MgCl_2$ (0.05 mM) was able to increase such activities 5 and 70 times, respectively. Based on their differential expression in the different morphological stages of *F. pedrosoi*, a possible role in this process was suggested. Since inhibition of ectophosphatase activity in cryptococci results in smaller rates of association of fungi with animal epithelial cells, it was proposed that ectophosphatase in *C. neoformans* may contribute to fungal colonization of the animal host. A cell wall-associated phosphatase has been detected in *F. pedrosoi* cell walls.[118] It was strongly inhibited by exogenous inorganic phosphate (P_i); on the other hand, removal of P_i resulted in a 130-fold increase of ectophosphatase activity. Conidia with high ectophosphatase activity showed greater adherence to mammalian cells than did fungi cultivated in the presence of P_i, suggesting a role in adhesion to host cells.

F. pedrosoi, *F. compacta*, *Phialophora verrucosa*, *Cladosphialophora carrionii*, *Cladophialophora bantiana* and *Exophiala jeanselmei* have urease, gelatinase and lipase activity.[246] Instead, only phospholipase was detected in *F. pedrosoi*, a result that prompted the authors to suggest phospholipase detection as a tool to differentiate this species from other agents of chromomycosis.[246] On the other hand, keratinases (but not elastase, lipase or DNase) produced by *Microspoum canis* have been proposed as a virulence factor[277] due to the strong correlation between high keratinase activity and the development of symptoms in samples isolated from symptomatic or asymptomatic dogs and cats.

Exocellular enzymes depend on a secretory system to carry them out of the cellular environment. Bernardo et al[22] studied the pre-vacuolar branch of exocytosis in *C. albicans*, and were able to identify structural homologs of several *S. cerevisiae* pre-vacuolar secretory genes, including the late-Golgi vacuolar protein sorting gene *VPS1*. *C. albicans VPS1* contains a 2082 bp intronless open reading frame whose deduced protein product is 73.3% similar to *S. cerevisiae* Vps1p and includes GTP-binding regions that are conserved in members of the dynamin-like GTPase family of proteins. *VPS1*$^-$ mutants lost their ability to secrete extracellular proteinases, and were incapable of producing filaments. Both facts are related to *C. albicans* virulence and therefore, the vacuolar system becomes an important element in the pathogenic process.

Genes involved in fungal cell wall synthesis

The fungal cell wall structure and its involvement in the dimorphic process has been a constant subject of research. In *P. brasiliensis*, several papers report the cloning, characterization and expression of genes such as β-1,3-glucan synthase,[201] α-1,3-glucan synthase and the regulatory small GTPase Rho2,[239] the chitin

synthase multigene family[182–185,225,228,231] and β-1,3-glucanosyltransferase.[50]

β-1,3-Glucan is a fungal cell wall polymer synthesized by the multi-subunit enzyme β-1,3-glucan synthase (FKS). The only FKS gene in *P. brasiliensis* (*FKS1*) has an open reading frame of 5942 bp, interrupted by two putative introns, and a deduced sequence of 1926 amino acids. *P. brasiliensis* Fks1p is a transmembrane protein.[201] Activation of β-1,3-glucan synthase in *P. brasiliensis* requires the participation of the *PbrRHO1* product as the GTPase regulatory subunit.[239]

The α-1,3-glucan synthase gene (*PbrAGS1*) presents six exons accounting for a putative coding region of 7293 bp, separated by five introns.[239] It encodes a predicted protein of 2431 amino acids, with a calculated mass of 274 kDa. It is expressed in the Y phase, where the polysaccharide is solely found. Comparison of the levels of expression of *P. brasiliensis AGS1* and *RHO2* in the M and Y stages of the fungus shows a direct correlation, suggesting a post-transcriptional regulation of *P. brasiliensis AGS1*, through the product of *RHO2*.[239] Also in this family is Cdc42, a pivotal molecule in establishing and maintaining polarized growth for diverse cell types, as well as during pathogenesis of certain fungi. Almeida et al[2] evaluated its role during cell growth and virulence of the yeast form of *P. brasiliensis* and found that the expression of *PbCDC42* in yeast cells promoted a decrease in cell size and more homogenous cell growth, altering the typical polymorphism of wild-type cells. Reduced expression levels also led to increased phagocytosis and decreased virulence in a mouse model of infection. Hence, Pbcdc42p seems to be an important protein during host-pathogen interaction, with special relevance to the polymorphic nature and cell size in the pathogenesis of *P. brasiliensis*.

The third important polysaccharide component of *P. brasiliensis* cell wall is chitin. It serves functions in strengthening the fungal cell wall and protection of the cell against lysis provoked by the internal turgor pressure; it also participates in the connection of capsular polysaccharides to the cryptococcal cell wall, forming soluble complexes with glucuronoxylomannan (GXM).[96] Cultivation of *C. neoformans* in the presence of an inhibitor of glucosamine 6-phosphate synthase resulted in altered expression of cell wall chitin. These cells formed capsules that were loosely connected to the cryptococcal wall and contained fibers with decreased diameters and altered monosaccharide composition. GXM, the major capsular component, is synthesized in cytoplasmic compartments and transported to the extracellular space in vesicles. Cytoplasmic structures associated to vesicular compartments and reticular membranes are in close proximity to the polysaccharide. GXM was generally found in association with the membrane of intracellular compartments and within different layers of the cell wall.[192] Analysis of extracellular fractions from cryptococcal supernatants by transmission electron microscopy in combination with serologic, chromatographic and spectroscopic methods revealed fractions containing GXM and lipids. These results indicate an intimate association of GXM and lipids in both intracellular and extracellular spaces consistent with polysaccharide synthesis and transport in membrane-associated structures.

GXM is also produced by species of the *Trichosporon* genus, i.e., *Trichosporum asahii*.[95] Trichosporal and cryptococcal GXM share antigenic reactivity, but *Trichosporum* polysaccharide has smaller effective diameter and negative charge. GXM anchoring to the cell wall was perturbed by dimethylsulfoxide and required interactions of chitin-derived oligomers with the polysaccharide. GXM from *T. asahii* supernatants are incorporated by acapsular mutants of *C. neoformans*, which renders these cells more resistant to phagocytosis by mouse macrophages. Despite similarities in cell wall anchoring, antigenic and antiphagocytic properties, trichosporal and cryptococcal GXMs manifested major structural differences that may affect polysaccharide assembly at the fungal surface.[95]

Chitin synthesis is controlled by a multigene family, some of them redundant. Based on differences in regions of high sequence conservation, chitin synthases have been organized according to their amino acid sequences into two domains and seven classes.[224] In *P. brasiliensis* six different chitin synthase genes have been identified.[182,183,265] *PbrCHS5* has a 5583 bp-long ORF, interrupted by three introns of 82, 87 and 97 bp. The deduced PbrCHS5 protein contains 1861 amino acids with a predicted molecular weight of 206.9 kDa.[183,185] Two domains are identified, one towards the N-terminal end of the protein (aa 16 to 786), with partial identity to myosin motor-like domains, and a second one towards the C-terminal end (aa 1221 to 1752) with homology to fungal chitin synthases. PbrChs4, while being a protein as large as PbrChs5, lacks sequences characteristic of myosin motors in its N-terminal region.[182,185] 5'UTR sequencing overlaps with a previously reported sequence containing the *CHS4* gene,[185] arranged in a head-to-head configuration with *CHS5*, in a similar fashion as *MDJ1* and *LON*,[19] mentioned in a previous section. *P. brasiliensis* CHS3 is the only one to have a higher expression in the yeast phase and at the end of the mycelium-yeast transition[15]; it contains a single ORF 3817 bp long with two introns (71 and 86 bp) encoding a 1220 amino acid polypeptide with high similarity to other fungal chitin synthases.

chs2 was chosen by Matute et al[152] to study background selection at the locus in *P. brasiliensis* species complex. For this, the DNA sequence for the *chs2* locus was determined in 67 samples. Of the 16 nucleotide substitutions located in the coding regions, 5 of them were synonymous and 11 non-synonymous. Because of the very limited levels of polymorphism within each one of the *P. brasiliensis* species and the low recombination levels observed in this region, the observed data could be more likely explained by the selective forces that affect loci over most of the chromosome, but at a considerable distance from *chs2*.

In other fungal species, efforts have been addressed mainly to cell wall-associated proteins and glycoproteins. In *C. albicans*, *S. cerevisiae* and *Y. lipolytica*, cell wall proteins were either labeled with biotin or radiolabeled with amino acids, and chased for a period of time representing several generations. No significant turnover took place during the chase period, and in fact radioactive proteins were accumulated in the wall during the period, indicating that proteins bound to the cell wall are stable and that there is no precursor-product relationship among those linked by non-covalent bonds and the covalently bound ones.[224] The composition, structure and synthesis of the cell wall of *C. albicans* display both subtle and important differences with the wall of different saprophytic fungi, of utmost importance for its pathogenic behavior (for a review, see Ruiz-Herrera et al[223]).

Important cell wall proteins are adhesins that help in host-pathogen interactions, inasmuch as adherence to target cells is a prerequisite for fungal dissemination and systemic complications. Adherence to extracellular matrix (ECM) proteins has been extensively studied in *S. schenckii*.[121,122,258] Early experiments with immobilized fibronectin[122] indicated that yeast cells and conidia adhered equally to the glycoprotein, in a dose-dependent manner; however, when the experiment was carried out with soluble fibronectin, conidia displayed a very low binding capacity compared to the yeast cells. This contradictory result may be the consequence of tridimensional modification of the protein structure, once this is subjected to an immobilization procedure that leads to modifications on the exposure of adhesive domains, as reported for other microorganisms.[121] *S. schenckii* binding to fibronectin may be associated to the classical tripeptide arginine-glycine-aspartic acid (RGD) adherence region of ECM molecules, a

conclusion derived from the fact that while *S. schenckii* binds to the RGD-containing 120 kDa fibronectin fragment, inhibition assays with RGDS and GRGDESP peptides did reduce adherence by 50% to soluble fibronectin,[121] while no reduction was observed when immobilized fibronectin was used.[122] Further research[258] aimed to correlate *S. schenckii* virulence with protein pattern of cell wall proteins and capacity to bind fibronectin indicated that no direct relationship between virulence and clinical or environmental clinical isolates. The lowest virulence was found in an isolate recovered from a patient with meningeal sporothrichosis. This isolate (IPEC 17943) exhibited the lowest capacity to interact with fibronectin, and showed only one fibronectin-binding protein, a 67 kDa variant of gp70 reported as a cell wall protein involved in fungal adherence to dermal extracellular matrix.[222] The most virulent isolates (IPEC 15383 and 1099-8, from disseminated cutaneous and osteoventricular, and subcutaneous sporothrichosis, respectively) showed a higher adhesive capacity, and expressed at least four fibrinogen-binding proteins (92, 55, 44, and 37 kDa) besides the 70 kDa band characteristic of gp70.[258]

H. capsulatum yeast-cell binding to glycosylated surface molecules of murine peritoneal or alveolar macrophages was studied using attachment inhibition assays with different carbohydrate-treated yeast cells.[81,256] Galactose (mainly as the β-anomer) and its derivatives were the most efficient sugar inhibitors. These results suggested the presence of a lectin-like component in *H. capsulatum* yeast cells and revealed involvement of galactosylated surface molecules of murine macrophages as specific-sugar (ligand) residues recognized by the fungal lectin. *H. capsulatum* yeast cells are also able to bind to erythrocytes irrespective of blood groups, an effect that could be inhibited not only by galactose but also by galactose-containing disaccharides and glycosaminoglycans, mainly chondroitin sulfate C, suggesting a possible association of the inhibitory effect with the presence of negative charges on the cell surface.[256]

In a histochemical study designed to evaluate the correlation between the adherence of *C. albicans* and *C. parapsilosis* to human buccal epithelial cells and the expression of fungal cell surface carbohydrates, Lima-Neto et al[123] found that adherence was higher in *C. albicans* than *C. parapsilosis*, and that individual strain differences correlated with a high content of α-L-fucose residues in cell surface glycoconjugates, suggesting that this monosaccharide may represent recognition molecules for interactions between the yeast and the host. In *C. glabrata*, host-pathogen interaction in vitro depends mainly on the adhesin Epa1, one of a large family of cell wall proteins. Most of the *EPA* genes are located in subtelomeric regions, where they are transcriptionally repressed by silencing. In order to better characterize the transcriptional regulation of the EPA family, Rosas-Hernández et al[221] assessed the importance of *C. glabrata* orthologues of known regulators of subtelomeric silencing (SIR2, SIR3, SIR4, HDF1 (yKu70), HDF2 (yKu80), and RIF1) in *S. cerevisiae*. They found that, whereas the SIR proteins are absolutely required for silencing of the reporter genes and the native subtelomeric EPA genes, the Rif1 and the Ku proteins regulate silencing at only a subset of the analyzed telomeres. A *cis* element adjacent to the EPA3 locus can silence a reporter gene when placed at a distance of 31 kb from the telomere.[221]

The cell surface of *C. albicans* and other ascomycetous yeasts is enriched in highly glycosylated mannoproteins that play roles in the interaction with the host tissues. As with other biological systems, *C. albicans* protein glycosylation occurs mainly through two distinct pathways, either O- or N-glycosylation. Examples of the former are the enzymes dolichol phosphate glucose synthase that catalyzes the transfer of sugar moieties from either UDP-Glc to dolichol phosphate glucose,[9] the corresponding mannose synthase, activated by cAMP-mediated protein phosphorylation,[10] and the mannosyl transferase.[11] Example of the latter is the N-glycosylation helped by α-1,2-mannosidase (MNS1), an enzyme involved in the hydrolysis of Man9GlcNAc2 and Man8GlcNAc2 oligosaccharides.[168] This goes by means of a post-translational modification initiated in the endoplasmic reticulum, where the Glc(3)Man(9)GlcNAc(2) N-glycan is processed by alpha-glucosidases I and II and alpha1,2-mannosidase to generate Man(8)GlcNAc(2), enzymes codified by *CWH41*, *ROT2*, and *MNS1*, respectively.[168] The N-oligosaccharide is then elaborated in the Golgi to form N-glycans with highly branched outer chains rich in mannose. Disruption of *C. albicans CWH41*, *ROT2*, and *MNS1* leads to mutants that tend to aggregate, have a lower content of cell wall phosphomannan and other changes in cell wall composition, and have a constitutively activated PKC-Mkc1 cell wall integrity pathway.[168] MNS1 is localized to the endoplasmic reticulum[169] and is converted into a cytosolic soluble enzyme with the help of the Kex2 protease.[167]

The extraction of isolated cell walls from the yeast phase of *S. schenckii* with SDS and separation of proteins by SDS–PAGE led to the identification of a periodic acid-Schiff (PAS)-reacting 70 kDa glycoprotein (Gp70) that was purified by elution from electrophoresis gels. The purified glycopeptide exhibited a pI of 4.1 and about 5.7% of its molecular mass was contributed by N-linked glycans with no evidence for O-linked oligosaccharides. It is uniformly distributed at the cell surface. Gp70 seems specific for *S. schenckii* as no immunoreaction was observed in SDS-extracts from other pathogenic and non-pathogenic fungi. Yeast cells of the fungus abundantly adhered to the dermis of mouse tails and the anti-Gp70 serum reduced this process in a concentration-dependent manner, suggesting Gp70 involvement in *S. schenckii* pathogenesis.[222]

Sialic acids have also been described as components of the fungal cell wall in several species, where they contribute to the negative charge of fungal cells, playing a role in their specific interaction with the host tissue. Back in 1998, Soares et al.[237] reported that sialic acid residues are major anionogenic groups exposed on the *P. brasiliensis* surface, joined to galactose by means of α-2,6- and α-2,3- links. Similar results were later reported when studying the cell-surface expression of sialic acids in two isolates of *C. albicans*.[238] Sialic acid reduces the binding of laminin and increases the binding of fibronectin to *S. schenckii* yeast cells.[121]

Melanin has been proposed as a virulence factor in fungi. Although not strictly a constituent element of the cell wall structure, when it is produced, it accumulates mainly within the cell wall mesh, giving cells and colonies a characteristic brown to black pigmentation. Melanin is synthesized by laccase enzymes, a group of multifunctional enzymes, in medium containing substrates such as L-dopa. To evaluate and compare laccase enzymes from clinical and environmental strains of *C. neoformans*, 30 Brazilian strains (15 clinical and 15 environmental isolates), belonging to serotypes A and B, were analysed.[200] All strains showed laccase enzyme activity; over half of the clinical strains of *C. neoformans* (56.2%) produced the lowest melanin intensities, suggesting that melanin production may not be the main virulence factor against host defence. Furthermore, virulence could not be associated with the origin of the sample, either clinical or environmental.

Fungal sphingolipids

Several glycosphingolipids (GSL) from different human pathogens have been characterized, and frequently involved in host-pathogen interaction. Fungi also present unique glycolipids which may have an important role for the fungal development and/or disease establishment. The different biological roles for GSL of different pathogens as infectivity factors and potential targets for development of new therapeutic strategies have been reviewed by Suzuki et al.[250]

Following studies on GSL in several dimorphic fungi, Takahashi's group[262–264] analysed their structure, composition, and dimorphic expression in *S. schenckii*. In lipids extracted from the mycelial phase, a single cerebroside (Cer) component (glucosyl-Cer) was observed, while in the yeast phase a galactosyl-Cer was also detected. It is worth noting that glucosyl-Cer and its corresponding synthase have been reported as a virulence factor in *C. neoformans*.[250] The major long chain core in all three cerebrosides was found to be (4*E*,8*E*)-9-methyl-4,8-sphingadienine, as reported for the majority of fungi.[263]

Glycosylinositol phosphorylceramides (GIPCs) are a class of GSL that appear to be essential for fungal survival. In *S. schenckii*, GIPC structures were determined to be Manα1→6Ins1-P-1Cer and Manα1→3Manα1→6Ins1-P-1Cer (where Ins = *myo*inosytol, P = phosphodiester) in the mycelial and the yeast phases.[263] An additional GIPC with the structure Manα1→3Manα1→6GlcNH$_2$α1→2Ins1-P-1Cer was reported in both phases.[264]

Acidic GSL components were extracted from *A. fumigatus* and identified as inositol phosphorylceramide and glycosylinositol phosphorylceramides.[261] The structures of six major components were elucidated as Ins-P-Cer, Manα1→3Manα1→2Ins1-P-1Cer, Manα1→2Manα1→3Manα1→2Ins1-P-1Cer, Manα1→3[Galβ1→6]Manα1→2Ins1-P-1Cer, and Manα1→3Manα1→6GluNα1→2Ins1-P-1Cer.[262] Similar glucosylceramide and galactosylceramide are present in *A. nidulans*, playing roles in germination and hyphal growth, as demonstrated by their inhibition when the fungus was treated with D-threo-1-phenyl-2-palmitoyl-3-pyrrolidinopropanol (P4) and D-threo-3',4'-ethylenedioxy-P4, belonging to a family of compounds known to inhibit GlcCer synthase in mammals.[120] *Pseudallescheria boydii*, a fungal pathogen that causes disease in immunocompromised patients, also synthesizes glucosylceramides as major neutral glycosphingolipids. Ceramide monohexosides are detectable on the surface of mycelial and pseudohyphal but not conidial forms of *P. boydii*, suggesting a differential expression of glucosylceramides according with the morphological phase. Addition of antiglucosylceramide antibodies to cultures of *C. albicans* clearly inhibited the generation of germ tubes, suggesting an involvement of ceramide monohexosides in differentiation and infectivity.[205] In *F. pedrosoi*, the main cerebroside species found in mycelia and conidial forms is N-2'-hydroxyhexadecanoyl-1-beta-d-glucopyranosyl-9-methyl-4,8-sphingadienine, while the major cerebroside species purified from sclerotic cells carries an additional hydroxyl group, bound to its long-chain base. The structural difference between cerebrosides from mycelial and sclerotic cells was apparently not relevant for their antigenicity, since they were both recognized at similar levels by sera from individuals with chromoblastomycosis and a monoclonal antibody to a conserved cerebroside structure.[180]

An interesting application of lipid biology to the clinics of PCM comes from the work of Bertini et al.[23] By enzyme-linked immunosorbent assay of sera from 31 PCM patients, these authors analyzed immunoglobulin classes and isotypes of antibodies directed to acidic glycosphingolipids (GSLs) and glucosylceramide of *P. brasiliensis*. Only the GSL Pb-1 antigen, which presents the carbohydrate structure Gal*f*-β1-6(Man-α1-3)Man-β1, was reactive with the PCM patient sera. The Gal*f* residue is essential for antibody reactivity, as shown by the lack of reactivity of Pb-2, the biosynthetic precursor of Pb-1, in which that sugar moiety is absent. The Pb-1 glycolipid from nontreated patients elicited a primary immune response with immunoglobulin M (IgM) production and subsequent switching to IgG1 production. The IgG1 titer increased after the start of antifungal treatment, and general decreases in the anti-Pb-1 antibody titers were observed after 5 months of treatment. These results suggested that the Pb-1 antigen has potential application as an elicitor of the host immune response in PCM patients.

Sphingolipids and cholesterol, as important components of the cell membrane, may be organized in membrane rafts that play an essential role in different cellular functions, including host cell-pathogen interaction. In *P. brasiliensis*, the involvement of epithelial cell membrane rafts in the adhesion process of the pathogen and activation of cell signaling molecules was demonstrated once the ganglioside GM1, a membrane raft marker, was localized at *P. brasiliensis*-epithelial cell contact sites; the inhibition of fungal adhesion to host cells pretreated with cholesterol-extractor (methyl-beta-cyclodextrin) or cholesterol-binding (nystatin) agents was additional proof of the interaction.[36,153]

Lipid rafts may also be involved in the trafficking of polysaccharide macromolecules from the cytoplasm to their final destination in the outer cell wall, without breaking apart membranes.[1,216,217] Recent reports indicate that extracellular vesicles, physiologically secreted across the cell wall, help in the export process not only of the major *C. neoformans* capsular polysaccharide glucuronoxylomannan (average mass, 1.7 x 10^6 to 7 x 10^6 daltons),[216] but also of a variety of virulence factors (e.g., glucosylceramides, laccase, urease).[216,217] Additionally, 76 vesicle-located proteins were identified by proteomic analysis, of which 27 had already been reported as vesicular proteins in mammalian exosomes.[217] Such vesicles are built with bilayered membranes containing key fungal lipids, such as GlcCer, and ergosterol, supporting the idea that they are enriched in lipid rafts, and conforming a sophisticated trans-cell wall vesicular transport secretory mechanism that is not available in prokaryotes; it may also indicate that extracellular vesicles function as "virulence bags" that deliver a concentrated payload of fungal products to host effector cells and tissues.[213,216,217] Analogous findings have been reported in *H. capsulatum*,[1] suggesting a general mechanism in fungi for the transport of virulence-related macromolecules through vesicular secretion. Additionally, the fact that similar vesicles have been found in species belonging in ascomycetes (*H. capsulatum*) and basidiomycetes (*C. neoformans*) may suggest that the shuttle system is ancient, predating the divergence of these branches 0.5-1.0 billion years ago.[189]

Transcriptome, genome, molecular taxonomy

Studies on the transcriptome of *P. brasiliensis* carried out by Felipe et al.[86] and Goldman et al[102] have revealed expressed sequence tags (EST) that could be organized in functional categories such as cellular metabolism, information storage and processing, cellular processes-cell division, posttranslational modifications, morphogenetically-linked genes, among others. Molecular techniques such as microarrays and substraction hybridization have allowed the identification of genes involved in basic and cell wall metabolism, sulfur metabolism, amino acid catabolism, signal transduction, growth and morphogenesis, protein synthesis, genome structure, oxidative stress response, and development genes that are preferentially expressed in the yeast phase,[18,143,190] or differentially expressed in host-fungus interaction.[13,65,254] Since conidia are more likely to be the infecting propagules, García et al.[98] studied this process and found sequences not previously described, which could represent novel exclusive conidia-yeast transition genes.

Two fungal species, *C. immitis* and *P. brasiliensis*, both strongly related to the Latin American region, have been the subject of extraordinary advances in molecular taxonomy and phylogeny. *C. immitis*, the etiologic agent of coccidioidomycosis, is endemic to arid soils of the American continent, principally the lower Sonoran life zone and desertic areas of Argentina and Venezuela.[93] Fisher et al,[93,94] in a continental joint effort led by John Taylor at the

University of California, Berkeley, were able to collect 161 clinical and two environmental isolates, covering the known geographical range of *C. immitis*. In them, allele distributions at the nine microsatellite loci were sampled from eight geographical populations. The resulting tree showed that isolates occur within one of two major clades, known as the Californian and non-Californian phylogenetic species; the latter was renamed *Coccidioides posadasii* to honour Alejandro Posadas, the Argentinean researcher who in 1892 reported the fungus for the first time. It may represent a divergent, genetically recombining monophyletic clade.[93,94] *C. posadasii* is the most frequent species in the northern-central region of Mexico.[49]

P. brasiliensis is confined to the Latin American region.[231] This fungus is considered clonal according to mycological criteria; at the same time, it shows extensive genetic variability when analyzed by molecular tools. RAPD analyses,[37] RFLP,[181] and partial sequences of some genes[112,166] from a high number of *P. brasiliensis* isolates, revealed genetic variability and clusters correlated with geography[37,181] or virulence.[45,165] Matute et al[149] analyzed *P. brasiliensis* phylogenetically in search of cryptic species and found that this fungus consists of at least three distinct, previously unrecognized phylogenetic species: S1 (species 1 with 38 isolates of assorted geographical origin), PS2 (phylogenetic species 2 with six isolates, five Brazilian and one Venezuelan), and PS3 (phylogenetic species 3, with 21 Colombian isolates). S1 and PS2 were sympatric across their range, suggesting barriers to gene flow other than geographic isolation. Variations in virulence and gene expression of antigenic proteins have been found between *P. brasiliensis* isolates now known to belong to species S1 and PS2.[112] Despite their differences, all three species are capable of inducing disease in both humans and armadillos.[45] Matute et al[151] also developed a marker system for DNA-based recognition of phylogenetic species S1 and PS2 in *P. brasiliensis*, based on microsatellites. Searching for positive selection in putative virulence factors, Matute et al[150] reported on the selection of 12 such genes involved in different cellular processes, either antigenic or involved in pathogenesis. Only two genes (*p27* and *gp43*) have unknown functions. All other genes were classified in four different categories: metabolically related (*fas2*, *his1*), cell wall related (*fks*, *mnn5*, *ags1*), heat shock proteins, detoxification related (*tsa1*, *sod1*, *hsp88*) and signal transduction (*cdc42*, *cst20*). Several replacement mutations in *gp43* were under positive balancing selection. The other three genes (*fks*, *cdc42* and *p27*) showed very little variation among the *P. brasiliensis* lineages and appeared to be under positive directional selection.

Following phylogenetic studies, Carrero et al[44] reported coding and non-coding regions from various genes and the ITS region in 21 isolates of *P. brasiliensis*, seven of them new. This study showed that the majority of the sequences used by Matute et al[149] and those used in this study, grouped within two (S1 and PS3) of the three clades proposed by these investigators. However, one *P. brasiliensis* isolate, Pb01, was placed at the base of, and quite distant from, the three species reported by Matute et al,[149] clustering together with strain IFM 54648, an atypical strain isolated from a patient in the southern Brazilian region of Paraná.[253] This finding suggested the possibility of more than three phylogenetic species in *P. brasiliensis*.[44] Further work[257] gave strength to this hypothesis, once the identification of 17 isolates, out of 88 samples, genotypically similar to strain Pb01, allowed their grouping as Pb01-like isolates. They are considered a new phylogenetic species distinct from the S1, PS2 and PS3 clades previously reported by Matute et al,[149] since it is strongly supported by all independent and concatenated genealogies, with highly significant values of posterior probability (1.0) and bootstrap agreement (100%). The speciation event that defined this new phylogenetic group is sympatric relative to S1 and PS2. The two separate groups that include S1, PS2, PS3 on one side and Pb01-like on the other, were highly divergent

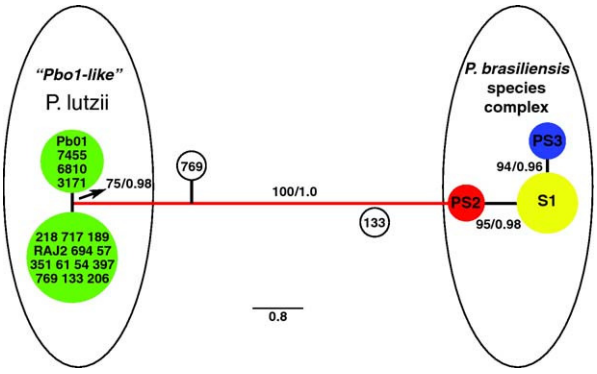

Figure 2. Bayesian unrooted phylogram showing the relationship between the isolates from the three phylogenetic species S1, PS2, PS3 and the isolates from the "*Pb01-like*" cluster. Eight concatenated loci, comprising 3,565 nucleotides, from dataset 1 (*fks*-exon2, fks-exon3, *chs2*-exon1, *chs*2-exon2-4, *gp43*-promoter-exon1, *gp43*-exon2, *arf* and α-tubulin). This is a consensus unrooted tree highlighting the distance that separate the three phylogenetic species of *P. brasiliensis* from the "*Pb01-like*" (*Paracoccidioides lutzii*) group. The scale means the numbers of substitutions per site analysed [modified from 255]. Reproduced by permission.

(fig. 2).[257] Based on molecular phylogenetic data, distinctive morphological characters and a long period of genetic isolation (> 30 million years) that set the two groups apart, the Pb01-like clade may be considered a new phylogenetic species, and we proposed the binomial name *Paracoccidioides lutzii*,[257] whose specific descriptor means to honour Adolpho Lutz, the Brazilian researcher who first reported *P. brasiliensis* in 1908.

Electrophoretic karyotypes of 12 clinical and environmental *P. brasiliensis* isolates from different geographic areas indicated the possible existence of haploid and diploid (or aneuploid) isolates of the fungus.[85] Further studies by flow cytometry and comparison with previous electrophoretic data[3] revealed a genome size ranging from 26.3 ± 0.1 Mb (26.9 ± 0.1 fg) to 35.5 ± 0.2 Mb (36.3 ± 0.2 fg) per uninucleated yeast cell in 10 *P. brasiliensis* isolates. The analysis of intra-individual variability of the highly polymorphic *P. brasiliensis gp43* gene[166] indicated that only one allele was present; therefore, all isolates presented a haploid, or at least aneuploid, DNA content; no association was detected between genome size/ploidy and the clinical-epidemiological features of the isolates.[3]

One extraordinary step forward in the field has been the recent public release of the genome of three *P. brasiliensis* strains, among them, the above mentioned Pb01 isolate, in an effort led by the Broad Institute, MIT, Boston, that included all Latin American laboratories involved in molecular biological research of the fungus (Brazil, Colombia and Venezuela), under the *Paracoccidioides* Comparative Genome Analysis Project. Data can be found at http://www.broad.mit.edu/annotation/genome/paracoccidioides_brasiliensis.2/MultiHome.html. Preliminary data-mining analyses indicate that the Pb01 strain does have important differences with the other two isolates, Pb18 and Pb03, particularly with regards to the genome size (32.94, 29.06 and 29.95 Mb, respectively) and number of genes (9132, 7875 and 8741 genes, respectively) [manuscript in preparation], a result that provides additional arguments in favour of the proposed classification of Pb01-like isolates as *P. lutzii*.

Despite their telomeric (sexual) stages or mating system being unknown, *C. immitis*, *C. posadasii*, *P. brasiliensis* and *P. lutzii* have been classified by molecular criteria as belonging to the phylum Ascomycota, Order Onygenales.[231] Recent work[267] aimed to determine the presence of the mating type locus in 71 *P. brasiliensis* isolates from various sources. Two heterothallic groups (MAT1-1 or MAT1-2) were recognized and, in some isolates, gene expression was confirmed, indicating the existence of a basal gene expression. The distribution of two mating type loci in the studied population

suggested that sexual reproduction might occur in *P. brasiliensis*. This finding points towards the possibility of applying a more precise definition of the concept of biological species to *P. brasiliensis*.

Beginning with the first reported human case of Jorge Lobo's disease, its etiologic agent, *Lacazia loboi*, has been at the center of a taxonomic dispute. The fungus was described as *Loboa loboi* but subsequent morphological, serological and molecular studies argued that *L. loboi* was a *Paracoccidioides* species.[279] To investigate the phylogenetic position of this species, Vilela et al[279] conducted a phylogenetic analysis using 20 *Lacazia loboi* isolates (as the species was renamed). To this effect, they used *L. loboi* DNA sequences from *ITS* rRNA, and partial coding sequences of *chitin synthase 4*, *ADP-ribosylation factor*, and *gp43* and compared them to those from 17 *P. brasiliensis* strains that represented the known variation in this species,[44,149,279] and outgroup taxa in the Onygenales (*Ajellomyces* and *Coccidioides* species). Nucleotide variation among strains of *L. loboi* was minor but numerous nucleotide mismatches and multiple gaps were found for these gene regions among members in the Ajellomycetaceae, including *P. brasiliensis*. Phylogenies inferred using neighbor-joining, maximum parsimony and Bayesian analyses depicted *L. loboi* as a well-supported, monophyletic group that was sister to the *Paracoccidioides* clade. The authors concluded that *L. loboi* should be maintained as a taxon independent from *Paracoccidioides* within the Ajellomycetaceae.[278]

Immunology

Innate immunity effector mechanisms in mycoses

One major development in the field of immunology is the acknowledgment that innate immunity, although not being highly specific, is able to efficiently detect microbial infections through the recognition of pathogen-associated molecular patterns (PAMPs) by specialized pattern recognition receptors (PRRs); membrane-bound Toll-like receptors (TLR), cytoplasmic nucleotide oligomerization domain-like receptors (NOD), dectin C, and others. These evolutionarily conserved structures are mainly present in monocytes, macrophages, dendritic cells, T and B lymphocytes, mediating the recognition of microbial pathogens and the subsequent inflammatory and immune responses.[158] In fungi, dectin-1, mannose receptor, TLR4, TLR2, and galectin-3 that recognize β-(1,3)-glucans, mannans, mannoproteins, phospholipomannan and β-mannosides, respectively, have been identified (reviewed by Gow et al[110]).

The simultaneous activation of multiple PRRs by a fungal pathogen directs the immune system to mount an ample and effective specific immune response against the fungus. Thus, the importance of PRRs and TLRs resides not only in directing the innate immunity but also in orchestrating the adaptive immunity developed in sequence. Many fungal wall components are recognized by host PPRs. González et al[108] showed that MyD88, an adaptor protein of TLR, is dispensable for resistance to *P. brasiliensis* and that TLR2, TLR4 and dectin-1 do not play a significant role in the recognition of *P. brasiliensis* yeast cells. However, further research[90] implicated TLR2 expression in susceptibility to this fungus. The group of Calich in Brazil has extensively studied the role of MyD88 and also of TLR2 and TLR4 in experimental paracoccidioidomycosis.[130] They demonstrated that TLR2 deficiency resulted in the development of milder infection, decreased nitric oxide synthesis and increased production of KC (murine analogous of IL-8), TGF-β, IL-6, IL-23, and IL-17.[38] Dectin-1, CD18, and TLR2 receptors are also involved in the lipid body formation induced by the cell wall β-glucan of *H. capsulatum*, a phenomenon linked to leukotriene B4 generation.[241] Studies with *C. albicans* showed that the interaction of this fungal agent with intrahepatic lymphocytes resulted in the up-regulation of TLR-2 expression in this cell population.[211] The involvement of TLR4 in the recognition of *S. schenckii* was suggested by studies that compared TLR4 deficient and sufficient mice and which showed that both pro-inflammatory and anti-inflammatory mediators were reduced in the TLR4-deficient group.[234] Therefore, the involvement of TLR4 in *S. schenckii* recognition by the host was first described by Latin American authors.

A critical point at the initial stage of antifungal defense is the production of chemotactic factors (cytokines, chemokines and leukotriens) at the site of the infection, for the effective recruitment of phagocytes (neutrophils, monocytes and macrophages), dendritic and natural killer cells. For instance, the cytokine IFN-γ modulates the chemokine production and leukocyte recruitment to the lungs of *P. brasiliensis*-infected mice.[248] On the other hand, *H. capsulatum* induced generation of high levels of MIP-1-α, and of low levels of eotaxin and its β-glucan cell wall component induced a little MIP-1-α but considerably higher concentrations of eotaxin, suggesting that chemokines and leukotrienes may play key roles in the inflammatory cell influx to *H. capsulatum* infection.[156]

The production of pro-and anti-inflammatory cytokines and chemokines during the early stages of mycotic infections decisively influences not only the inflammatory response that is mounted shortly after the infection but also enhances or impairs the subsequent development of an effective protective immune response, being therefore decisive to the outcome of the subsequent disease developed. For this reason, the effect of each fungal agent on the production of different types of these mediators was the object of study of many Latin American authors.

Some of these results strongly suggest that an imbalance in the production of pro-inflammatory and anti-inflammatory cytokines may be associated with the pathogenesis of some mycoses. Peraçoli et al[199] have characterized the cytokines produced by monocytes from PCM patients and were able to demonstrate that endogenous levels of TNF-α, IL-1β, IL-6, IL-8, IL-10 and TGF-β, detected in monocytes from patients, were significantly higher than those produced by healthy controls. IL-18, a recently described cytokine, important in the regulation of both innate and acquired immune response, was also studied in PCM. The results showed that IL-18 knockout (IL-18 -/-) BALB/c mice were more resistant to *P. brasiliensis* than their wild type controls.[195] Mamoni and Blotta[138] showed that gene expresión, kinetics of cytokines and chemokines distinguishes *P. brasiliensis* infection from disease, as deduced from the earlier and higher levels of TNF-α, IFN-γ and chemokines mRNAs in PCM-infected patients as compared with patients suffering from the juvenile form of the disease. Preferential induction of pro-inflammatory cytokines was also demonstrated at the onset of experimental *P. brasiliensis* infection.[107] Patients with chromoblastomycosis produce high levels of IL-10 and low levels of IFN-γ, resulting in the development of a somehow impaired immune response.[244]

Phagocytes, (neutrophils and macrophages), which constitute the first cell population to confront the fungus after infection, have an important role, not only in the events directly related with fungal lysis but also with the activation of acquired immunity. Macrophages are activated by different mechanisms in order to perform their function with increased efficiency. Receptors for complement system components, mannans and β-glucan (like dectin 1) activate different pathways to make sure that fungi are engulfed by the phagocytes. These aspects were studied by Jiménez et al.[115] in relation with complement and mannose receptors. Employing congenic murine bone-marrow-derived macrophage lines infected with *P. brasiliensis* conidia, the authors suggested the participation of mannose receptor in phagocytosis and a major activating effect on the antifungal activity of these cells by cytokines, mainly IFN-γ.

Human phagocytes were also studied in this mycosis. Monocytes obtained from PCM patients, were preactivated with recombinant IFN-γ and evaluated for their fungicidal activity against *P. brasiliensis.* Cells from healthy subjects failed to present such activity while those from PCM patients showed significant fungicidal activity against virulent Pb18; in contrast, both patient and control cells were significantly fungicidal against avirulent Pb265.[39] Some factors can also inhibit phagocytosis: monocytes treated with indomethacin exhibited an effective killing *P. brasiliensis*, suggesting a role of prostaglandin E2 (PGE2) in the inhibitory process. Human monocytes challenged with the fungus produced high PGE2 levels, which in turn repressed the fungicidal activity by reducing H_2O_2 and TNF-α production.[31] Some fungi are able to secrete products that alter the fungicidal properties of macrophages. Indeed, a lipid component of the *S. schenckii* cell wall was shown to inhibit phagocytosis by macrophages.[43]

Apoptosis induction may lead to the impairment of some cells that play essential roles in the establishment of protective immunity against fungal infections. Peritoneal macrophages co-incubated with *C. albicans* strain CR1 *in vitro* show early signs of apoptosis, but evolve to necrosis after 2 h. At the same time, an increase in IL-10 production is observed. Treatment of CR1 with pepstatin (a proteinase inhibitor) prevented the process of apoptosis and significantly reduced IL-10 production, suggesting that the increased production of this cytokyne was caused by processes occurring during the initial phase of infection, such as apoptosis, necrosis and uptake of death cells.[99] The liver constitutes the first barrier in the control of hematogenous dissemination of *C. albicans* of intestinal origin. Renna et al.[211] studied the involvement of apoptosis and pro-apoptotic signals in the hepatic injury during the acute phase of *C. albicans* infection and concluded that in the scenario of early liver injury, the recruited intrahepatic lymphocytes and the modulated expression of TNF-α, Fas-L and TLR-2 molecules could act coordinately in delivering death signals.

Neutrophils are also deeply affected by cytokines, which can enhance or impair their phagocyitic activity. IFN-γ, as well as the more recently studied cytokine IL-15 facilitate *P. brasiliensis* killing by human neutrophils, in contrast to the effect of IL-10.[12,64,255] Using an experimental model of candidosis, it was shown that neutrophils, at a first phase and later, macrophages, are involved in clearing an experimental infection by *C. albicans*.[131] *F. pedrosoi* experimental inoculation elicited marked neutrophils migration to the inflammatory site followed by microbicidal activity, particularly against hyphae, suggesting that host resistance to this fungus is primarily mediated by neutrophils. As a high number of destroyed conidia was found intracellularly in macrophages, the further participation of these cells is suggested.[132]

The destruction of phagocytes through induction of apoptosis has been described in this review as a mechanism of fungal escape from immune response. *H. capsulatum* is a facultative intracellular parasite, found in neutrophils and mononuclear cells, suggesting that it is capable of evading damage and surviving inside these cells. The work of Medeiros et al[155] shows that *H. capsulatum*-infected leukocytes presented less apoptosis than controls, suggesting that this fungus induces an antiapoptotic state on neutrophils and monocytes. This phenomenon may represent an extraordinary escape mechanism, by delaying cell death and allowing *H. capsulatum* to survive inside phagocytes.

Macrophages and dendritic cells (DCs) are antigen-presenting cells with a fundamental role in connecting the innate and the acquired immune responses. DCs in particular have been recently recognized as initiators and modulators of immune responses and their role in mycotic infections constitutes a major area of research. After fungal infection, immature DCs are recruited to the inflammation site and transformed into mature DCs. They recognize fungal cells by their TLR receptors, and are induced to produce proinflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8 and IL-15. Cytokines, as well as co-stimulatory molecules are needed to render lymphocytes fully activated and so trigger acquired immune response.

The role of DCs is essential in subcutaneous mycoses, such as chromoblastomycosis, because its causative agent, the fungus *F. pedrosoi* and DCs have plentiful opportunity to interact at the onset of the infection. DCs from patients exhibited an up-regulated expression of human leucocyte antigen D-related (HLA-DR) and of co-stimulatory molecules (CD86). In the presence of conidia, the expression of HLA-DR and CD86 was up-regulated by DCs from patients and also from controls.[245] In one of the rare immunological studies in dermatophytosis, it was shown that *T. rubrum*-infected macrophages have down-regulated expression of co-stimulatory molecules (CD80 and CD54) and that the ingested conidia grow and differentiate into hyphae inside macrophages, leading to rupture of the cell membrane, suggesting the existence of mechanisms that evade or suppress protective immunity.[41] The expression of co-stimulatory molecules was also studied on T-cells and monocytes of active PCM patients and healthy individuals cured of past PCM. CD28 expression, critical for optimal T-cell activation, was comparable between patients and controls, whereas CD152, PD-1 and ICOS, which preferentially deliver negative signaling, were over expressed on stimulated and unstimulated T-cells from patients. CD80 and CD86 were equally expressed on monocytes from patients and controls, but over expressed in T-cells from patients.[36]

The recognition of fungal cells by the innate immunity leads to the immediate mobilization of effector and regulatory mechanisms that have as a consequence the formation of an inflammatory environment for the recognition of the pathogen, the mounting of the first line of defense that controls the fungi during the establishment of the acquired immune response, leading to the activation of either cellular or humoral immune response.

Mechanisms of humoral immunity–Role of specific antibodies in mycoses

During the last decade it has been demonstrated that, although not as determinant as cellular immunity, humoral immune response is also important in the mounting of an adequate immune protection against fungal infections, provided that protective antibodies be present in adequate concentration.[46,207] Although the protective role of specific antibodies against *P. brasiliensis* is still controversial, the contribution of Taborda's group was essential for the understanding of the humoral arm of anti-fungal immunity. In fact, Buissa-Filho et al[35] studied the effects of monoclonal antibodies to the major *P. brasiliensis* antigen (gp43) using *in vitro* and *in vivo* infection models. As reported by the authors, the passive administration of monoclonal antibodies against gp43 before and after infection led to a reduced fungal burden and decreased pulmonary inflammation, associated with enhanced phagocytosis of *P. brasiliensis* by macrophages, and increase in nitric oxide production by these cells.

In a series of studies on mycotic patients, several Latin American authors detected patterns of preferential isotypes production according to the clinical manifestations of each mycosis. For instance, Biselli et al[27] found that 100% of patients with the severe acute form of PCM produced high levels of anti-gp43 antibodies of IgE, an isotype which is preferentially synthesized in a Th2 cytokines environment, whereas only 27% of the patients with the chronic form of the disease produced this isotype at high levels. These results support the notion that the relatively more severe impairment of cellular immunity in the acute form of PCM is probably related to a Th2 pattern of immune response.

Patients with other mycoses were also the object of such studies. The IgG, IgM, and IgA immunoglobulins in sera of patients with sporotrichosis before antifungal treatment and also from patients with sporotrichosis during itraconazole treatment were quantified. More than 95% of patients had detectable IgA antibodies, and more than 85% had IgM and IgG antibodies before treatment. The number of patients with IgG antibodies increased to 91% during treatment. Conversely, significantly fewer samples from treated patients were positive for IgM (71%) and IgA (89%). Overall, 78% of patients had detectable levels of all isotypes tested at diagnosis, and this percentage dropped to 62.9% in patients that were receiving itraconazole.[5]

The majority o f substances that elicit good immune responses are proteins. They need to be processed and presented to T lymphocytes by antigen presenting cells (APCs) and are called T-dependent antigens. There are, however, some carbohydrate antigens that can be recognized as native molecules by B lymphocytes; these are the T-independent antigens. The fungus *C. neoformans* has a polysaccharide capsule composed of glucuronoxylomannan (GXM), which is such an antigen. Parra et al[197] quantified the production of IgG subclasses specific for GXM intervals after *C. neoformans* infection in moderately resistant (Balb/c), highly resistant (CBA/j) and susceptible (C57BL/6) mouse strains. Early production of IgG1, described as protector antibodies, coincided with a decrease of the number of *C. neoformans* colony forming units in the lungs.

Some fungi are able to produce and secrete products that interfere with components of the immune response, constituting, eventually, fungal virulence factors. Antigenic preparations from *S. schenckii* yielded proteases that are were able to cleave different subclasses of human IgG.[68]

The knowledge that antibodies against fungi could be protective led to the development of a very original approach by a group of Latin American and Italian researchers. They tested the *in vitro* fungicidal and the *in vivo* therapeutic activity of an engineered synthetic decapeptide was derived from the sequence of a recombinant anti-idiotypic antibody, that represents the internal image of a *Pichia anomala* killer toxin. This compound markedly reduced the fungal load in organs (liver, lung, spleen) of mice infected with *P. brasiliensis*, opening a new field in the induction of protective immunity against fungi.[269]

Mechanisms of cellular immunity–Role of lymphocytes and cytokines in mycoses

There is evidence of efficient activation of cell-mediated immunity after exposure to fungi. Lymphocytes from healthy subjects show strong proliferative responses against fungal antigens, producing numerous cytokines. In many mycotic diseases, the efficient tissue response against fungal invasion is a granulomatous inflammatory response, characteristic of cellular immunity. Resistance against fungi is based on a triple response, i.e., induction of a strong cellular immune response mediated by T helper lymphocytes with CD4 phenotype, production of cytokines and action of effector phagocytes (fig. 3).

The necessity of a strong, functional cellular immune response is illustrated by chronic mucocutaneous candidosis patients, who present defects in the cellular immunity. Peripheral blood mononuclear cells from these patients produced lower levels of IFN-γ and IL-2 than controls in response to *Candida* antigens, but did not produce higher levels of IL-4 and IL-10, suggesting that, even though Th1 cytokines are decreased, the Th2 response is not increased in this severe form of candidosis.[36] In cryptococcosis, however, the presence of capsular polysaccharidic components induce a dominant Th2 pattern, with high levels of IL-4 and IL-10 production and undetectable inflammatory cytokines, such as TNF-α and IFN-γ, constituting a powerful virulence factor.[4]

Corbellini et al[61] studied the delayed-type hypersensitivity response developed against *F. pedrosoi* exoantigens in 16 male guinea pigs, all but one showing positive response 48 h after inoculation, results that show that a specific T cell response, develops after exposure to chromoblastomycosis. The combined effects of both $CD4^+$ and $CD8^+$ T lymphocytes and Th1 and Th2 cytokines are required for the induction of resistance to various fungi. Chiarella et al[57] demonstrated in a murine model that $CD8^+$ T cells were the major elements involved in the control of *P. brasiliensis* loads, whereas $CD4^+$ T cells were responsible for delayed type hypersensitivity responses and antibody production. Teixeira de Souza et al,[259] infecting mice deficient in CD4 and CD8 T cells with *F. pedrosoi* showed that absence of CD4(+) cells induces a more severe disease in chromoblastomycosis.

Th1 lymphocytes produce predominantly IFN-γ and elicit phagocyte activation leading to respiratory burst. In contrast, Th2 lymphocytes synthesize predominantly IL-4 and IL-10 and promote the synthesis of antibodies, resulting in susceptibility to fungal infections and allergic reactions. Some fungi subvert the Th1/Th2 dicothomy in their favor. *C. neoformans* GXM profoundly alters the immune response, being responsible for many immunomodulation phenomena. It was shown that it suppresses lymphoproliferation in response to either concanavalin A or heat-killed *C. neoformans*, modulates cytokine production, determining high production of IL-10, and low secretion of IL-2, IFN-γ and TNF-α and also triggers macrophage apoptosis through NO generation.[55,56] The capsular polysaccharides, galactoxylomannan (GalXM) and glucuronoxylomannan (GXM) induced different cytokines profiles in macrophages. GalXM induced production of TNF-α, NO and iNOS expression, while GXM induced predominantly TGF-β secretion, but both induced macrophages apoptosis mediated by Fas/FasL interaction. All these phenomena constitute mechanisms by which capsular polysaccharides from *C. neoformans* might compromise host immune responses.[280]

High levels of IL-10 and TNF-α in the sera of chronic PCM patients have been reported.[97] Marques-Mello et al.[144] associated IL-4 and IL-5 production with a Th2 immune response to *P. brasiliensis* infection. Romano et al. showed that PCM-related Th1 immunosuppression was associated with down-modulation of the IL-12 pathway, and that patients cured from PCM may not fully recover their immune responsiveness.[220] The group of Blotta characterized the immune response of PCM patients suffering from clinically different manifestations of the disease: in the adult forms, the immune response is heterogeneous, with balanced Th1 and Th2 responses, preferential production of antibody isotype IgG1 in unifocal, milder cases, or IgE and IgG4 in multifocal severe cases; in the juvenile form, instead, Th2 response dominates, with production of IgA, IgE and IgG4 antibodies.[139] They also associated IL-18 and TNF receptor 2 with the severity of the disease.[62] In experimental models, it was demonstrated that the absence of functional IL-12 determines severe PCM in mice[125] and that this cytokine protects mice against disseminated infection but enhances pulmonary inflammation,[12] suggesting that resistance to *P. brasiliensis* infection correlates with preferential Th1 immune response.[117]

This may also be the case in other mycoses. Severe forms of chromoblastomycosis are characterized by the production of high levels of IL-10 and TNF-α, associated to low levels of IFN-γ and lymphocyte proliferation, in contrast with the mild cases, in which low levels of IL-10, high levels of IFN-γ and good lymphocyte proliferation were observed.[154] It has been described that immune modulation with recombinant IL-12 or anti-IL-10 can restore the antigen-specific Th1-type immune response in chromoblastomycosis patients by up-regulating HLA-DR and co stimulatory molecules in monocytes.[244] Zaga-Clavellina et al[285] reported that *C. albicans* induced differential synthesis and secretion of IL-1β, IL-6 and prostaglandin-E.

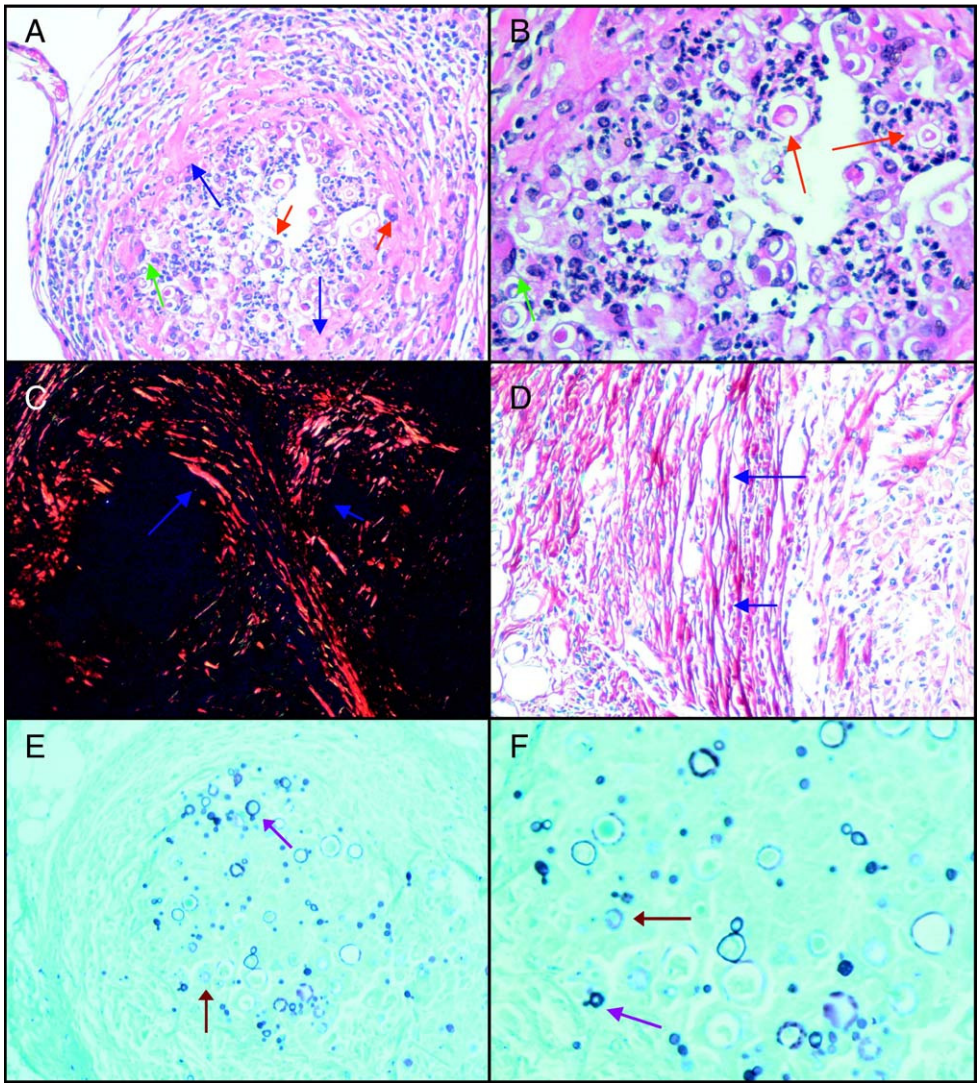

Figure 3. Histological analysis of granulomatous lesions in B10.A mice, susceptible to *P. brasiliensis*. The organ was collected 15 days after IP infection with the highly virulent Pb18 isolate. This figure illustrates the close interaction of different cell populations with the immune response in the course of experimental infection. A.Compact granulomatous lesion with presence of yeast cells, inflammatory cellular influx and extracellular matrix components. Magnification x200; HE dye. B.Marked influx of phagocytic cells of different lineages, some showing ingested *P. brasiliensis* cells. Magnification ×400; HE dye. C. Concentric collagen fibers circumscribing the granuloma and eventually containing fungal dissemination. Magnification x200; Sirius red dye observed under Polarized light. D. Marked deposition of thick collagen fibers delimiting the lesion. Magnification x400; Sirius red dye. E. *P. brasiliensis* at the center of the lesion. Magnification x200; Groccot dye. F. *P. brasiliensis* with preserved or altered morphology. Magnification x400; Groccot dye. Extracellular matrix components (blue arrows), neutrophils (green arrows), multinuclear giant cells (red arrows), fungi with preserved (violet arrows) or altered (brown arrows) morphology. Figure authors: RFS Molina and E. Burger. Original figure for this manuscript.

Da Silva et al[69] demonstrated that Langerhans cells were able to phagocytose *F. pedrosoi* conidia but not sclerotic cells, inhibiting hyphae formation. The development of local immune responses at the site of the granulomatous lesions and the interaction of fungi with extracellular matrix components and cytokines was studied in *P. brasiliensis*[106,187,188] and *S. schenckii* models.[91]

The role of nitric oxide (NO) in the fungicidal effect of macrophages has been a recurrent subject of research. Macrophages represent the major cell defense against fungi; when activated with IFN-γ, they are fungicidal by an oxygen-independent mechanism via the enhanced production of NO. However, the role of NO in mycosis is much more complex and the data obtained with studies of various fungi, albeit numerous, are mostly inconclusive.

One example is the persistence of *Cryptococcus* in the central nervous system in spite of marked local expression of mRNAs of nitric oxide synthase (iNOS) and stimulatory cytokines: increased levels of transcripts corresponding to IL-1, TNF-α and iNOS were detected as early as day 1 post infection, with TNF-α rising by approximately 30-fold and iNOS increasing by approximately 5-fold by day 7.[134]

In PCM an inverse correlation between NO concentration and transformation of *P. brasiliensis* conidia was observed.[105] Nishikaku et al[186] showed that NO has an important role in granuloma modulation, by controlling OPN and MMP production, as well as by inducing loose granulomas formation and fungal dissemination, resulting, at later phases, in PCM progression, thus confirming earlier results by Nascimento et al,[177] who described the dual role of this gas in experimental PCM. Moreira et al,[170] in turn, were able to show an association of IFN-γ or TNF-α-activated macrophages with higher levels of H_2O_2 and NO when compared to non-activated cells, an effect reversed with the addition of inhibitors. The role of nitric oxide synthase (iNOS) in PCM has also been studied by Livonesi et al[124] who suggest that iNOS is a resistance factor in PCM by controlling fungal proliferation, influencing cytokines production, and appeasing the development of a high inflammatory response and concurrent necrosis.

NO was found to be fungicidal against *S. schenckii*. Mice defective in the production of reactive nitrogen intermediates (iNOS-/- mutants) or wild-type mice treated with an iNOS inhibitor were used to investigate the role of endogenous NO during systemic sporotrichosis. The results suggest that although NO was an essential mediator to the *in vitro* killing of *S. schenckii* by macrophages, the activation of NO system *in vivo* contributes to the immunosuppression and cytokine balance during early phases of infection with this fungus.[87] Using *in vivo* and *in vitro* models of chromoblastomycosis, Bocca et al[30] demonstrated that, during the infection, *F. pedrosoi* peritoneal macrophages show an increased phagocytic capacity and H_2O_2 production, but also a reduced ability to produce NO, suggesting that inhibition of macrophages NO synthesis by the fungus-produced melanin could be partially responsible for the host's inability to eliminate *F. pedrosoi*, leading to the development of chronic disease.

The overall contribution of these and other works, led to the following concepts regarding cellular immunity in fungal infections: The production of IFN-γ is regulated by cytokine IL-12, considered to be the primary inducer of the inflammatory response. Deficiency in IL-12 and IFN-γ leads to extremely severe fungal diseases due to the inability of the host to liberate activation signals to effector phagocytes. On the other hand, the cytokine IL-4 is the most potent autocrine signal for commitment to Th2 reactivity, negatively modulating the protective Th1 responses, although higher susceptibility to mycotic infections is not always associated with increased production of IL-4. Many clinical observations suggest an inverse correlation between IFN-γ and IL-10 production in mycotic patients.

Vaccines

Among viral, bacterial, and fungal diseases, the latter are the only branch of infectious diseases without a vaccine for any of their causative agents. This is at odds with a disease burden that remains unabated by conventional chemotherapy and infection control measures.[48]

Using cell-free antigens (CFAgs), potential candidates to be developed as vaccines against *H. capsulatum* have been tested in murine models.[227] CFAgs not only induced a more potent delayed-type hypersensitivity response in *H. capsulatum*-infected mice than did histoplasmin, but also stimulated spleen cells from immune mice to produce higher amounts of IFN-γ in vitro, and protected against a lethal inoculum of *H. capsulatum*. In fact, yeast cells of *H. capsulatum* led to death in 83% of non-immunized mice after 45 days of I.V. infection, contrasting with 100% survival of CFAg-immunized mice; furthermore, intratracheal infection (the natural route of infection in humans) induced death of non-immunized mice after 18 days, whereas 72% of those immunized with CFAgs survived until the end of the 60-day postinfection observation period. Such induced protective immunity was reflected in a reducing fungal burden in lung and spleen. Protection of wild-type mice immunized with cell-free Ags from *H. capsulatum* against histoplasmosis was associated with increased leukotriene B4 and IFN-γ production as well as recruitment of memory T cells into the lungs.[157] In contrast, CFAg-immunized mice lacking 5-lipoxygenase(-/-), a critical enzyme involved in leukotriene synthesis, displayed a marked decrease on recruitment of memory T cells to the lungs associated with increased synthesis of the anti-inflammatory cytokine TGF-β and the Th1-related cytokine IL-10. These effects were associated with increased mortality to 5-lipoxygenase(-/-)-infected mice. In this way, an important immunomodulatory role of leukotrienes is established, in both the primary and secondary immune responses to histoplasmosis.[157] To improve on the method of vaccination, CFAgs have been encapsulated into biodegradable PLGA (poly(D,L-lactide-co-glycolide) microspheres (MS) that could allow the controlled and/or sustained release of the encapsulated antigens from *H. capsulatum*.[80]

The use of peptides as therapeutic vaccine adjuvants to chemotherapy in *P. brasiliensis* is an approach used by Luiz Travassos's group[142] in São Paulo, Brazil. For this, they used the main *Paracoccidioides* diagnostic antigen, gp43, secreted exocellularly by the infective yeast phase, as their source of potential vaccines.[251] The T-cell epitope of this antigen was mapped to a 15-amino-acid peptide (P10), in which 12-mer or longer sequences were active, confirming presentation by major histocompatibility complex II. The HTLAIR P10 inner core was the essential domain of the epitope. Immunization of mice with both gp43 and P10 led to vigorous protection against intratracheal challenge by virulent *P. brasiliensis*, with a >200-fold decrease in lung colony forming units (CFU) and no dissemination to spleen and liver. The protective effect of P10 was mainly attributed to an IFN-γ-mediated cellular immune response and not to the humoral (Th-1 and Th-2 activation) response seen with gp43.[251] To improve P10 delivery, these researchers synthesized a multiple antigen peptide with the protective T-cell epitope expressed in a tetravalent 13-mer analog of P10 (M10). M10 significantly protected intratracheally infected mice.[141,252] This research group also evaluated new anti-*Paracoccidioides* vaccine formulations based on the intranasal administration of *P. brasiliensis* gp43 or the P10 peptide in combination with the *Salmonella enterica* FliC flagellin, an innate immunity agonist binding specifically to the Toll-like receptor 5 (TRL5), in a murine model.[33] Mice immunized with recombinant purified flagellins genetically fused with P10 at the central hypervariable domain, or the synthetic P10 peptide admixed with purified FliC, elicited a prevailing Th1-type immune response based on lung cell secreted type-1 cytokines, and reduced *P. brasiliensis* growth and lung damage, suggesting a modulation of *S. enterica* FliC flagellin in the immune response to *P. brasiliensis* P10 antigen (fig. 3).

Using another approach, Reis et al[209] cloned a cDNA coding for an antigenic protein (Pb27) from *P. brasiliensis*. Mice immunized with purified recombinant Pb27 (rPb27) were able to develop high levels of IgG2b, moderate levels of IgG1 and low levels of IgG2a. At the same time the levels of TGF-β and IFN-γ were high while a very low production of IL-10 was verified. Using confocal microscopy with anti-rPb27 mouse serum against *P. brasiliensis* yeast forms, surface and cytosolic staining pattern were observed. Immunization of mice with this antigen induced a significant degree of protection in the lungs (93%), liver (93%) and spleen (100%) at 60 days after challenge with infection. Thus, the granulomatous lesions revealed a greater degree of compaction and organization, with few lesions in the lungs and no dissemination of the fungus to other organs. These results showed that rPb27 promoted acquired protection against infection with *P. brasiliensis* yeast forms, suggesting the use of this protein for future development as a prophylactic vaccine against PCM.

Glycosylceramides (GlcCer; see section 3.3) are immunologically active components inducing the production of antifungal antibodies. Nimrichter et al[179] purified and characterized a major GlcCer from mycelial forms of *F. pedrosoi*, the most frequent causative agent of chromoblastomycosis. By fast atom bombardment mass spectrometry (FAB-MS) analysis, the purified molecule was identified as N-2'-hydroxyhexadecanoyl-1-beta-D-glucopyranosyl-9-methyl-4,8-sphingadienine. A monoclonal antibody against this structure was used in indirect immunofluorescence with the different morphological stages of *F. pedrosoi*. Only the surface of young dividing cells was recognized by the antibody. Treatment of *F. pedrosoi* conidia with the monoclonal antibody against GlcCer resulted in a clear reduction in fungal growth. In addition, the monoclonal antibody also enhanced phagocytosis and killing of *F. pedrosoi* by murine cells, results that point to a possible

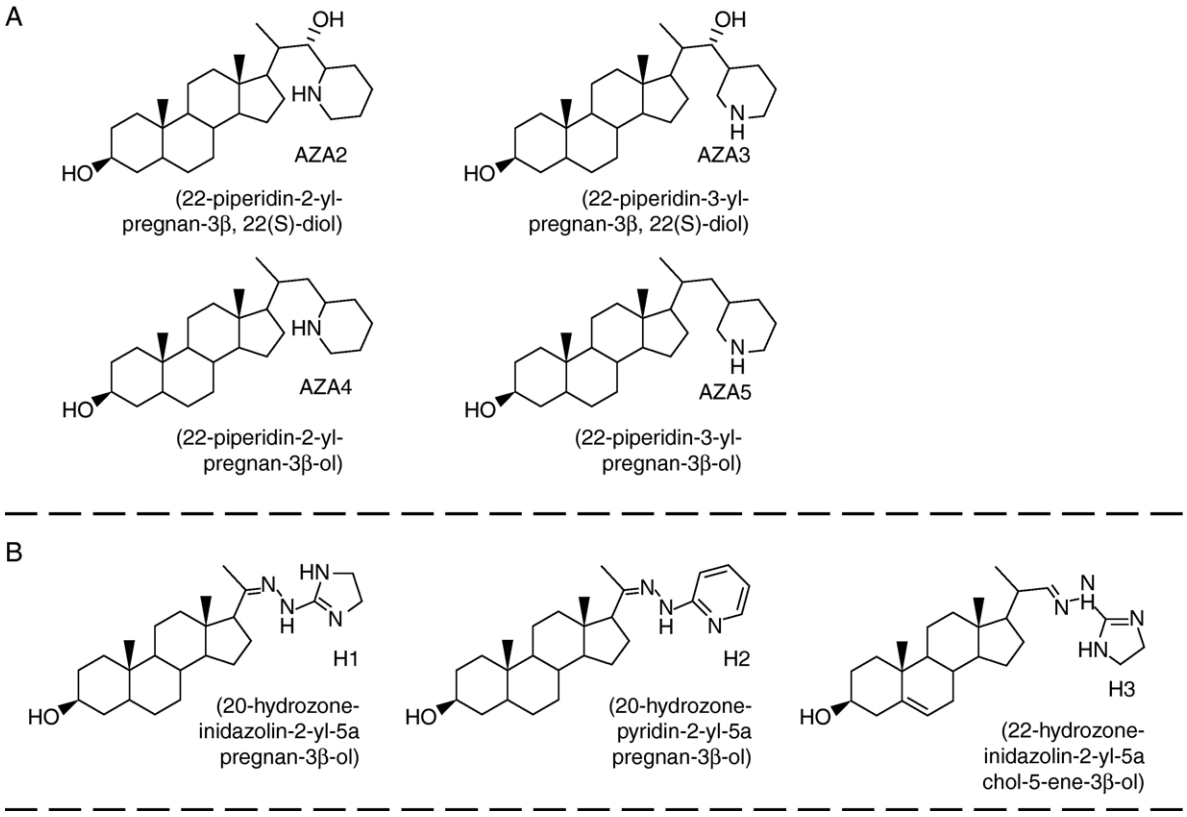

Figure 4. Chemical structures of selected azasterols (A) and sterol hydrazones (B). Structures provided by Gonzalo Visbal, Center of Chemistry, Instituto Venezolano de Investigaciones Científicas, Caracas, Venezuela.

use of monoclonal antibodies to GlcCer as potential tools in antifungal immunotherapy. Using *C. neoformans* GlcCer, Rodrigues et al[218] proved that passive immunization with a monoclonal antibody to GlcCer significantly reduced host lung inflammation and prolonged the survival of mice lethally infected with *C. neoformans*, revealing a potential therapeutic strategy to control cryptococcosis.[218] In the presence of the antibodies to GlcCer, inflammatory responses were better controlled by the host, resulting in reduced damage to host tissues and more effective killing of *C. neoformans* by host effector cells. In fact, the reduced inflammation in mice treated with the MAb to GlcCer corresponded to the increased lung concentrations of IL-4 on day 1 postinfection and anti-inflammatory cytokines, such as IL-4 and IL-6, on day 7.

Search for new antifungals

The rational design of new experimental antibiotics becomes an important tool to approach the search for new and more effective chemotherapeutic agents against fungal pathogenic species. Latin American researchers have been particularly active in this area. Many years ago, ajoene [(E,Z)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide], a platelet aggregation inhibitor chemically derived from garlic,[7] was reported to have antifungal effects against *P. brasiliensis*,[233] *A. niger* and *C. albicans*,[284] *Cladophialophora carrionii* and *F. pedrosoi*.[202] The antiproliferative effects of ajoene in *P. brasiliensis* were associated with a marked reduction in the content of phosphatidylcholine, with a concomitant increase in the levels of its precursor phosphatidylethanolamine, and a large increase in the amounts of unsaturated fatty acids in the Y phase.[233] A recent report[137] revealed that ajoene is capable of controlling the evolution of intraperitoneally induced PCM in Swiss mice, significantly reducing the levels of antibodies from the 10th week of treatment onwards. Ajoene therapy is also effective in association with sulfametoxazol/trimethoprim, showing a positive additive effect in mice intratracheally infected with *P. brasiliensis*,[260] expressed through the development of Th1-type cytokine responses producing higher levels of IFN-γ and IL-12 when compared to the infected but untreated members of the control group. Therefore, the antifungal activity of ajoene involves not only a direct effect on fungi but also a protective pro-inflammatory immune response.

The metabolic pathway to membrane sterols has been an all-time favourite in the search for selective antifungals. The pathway is blocked by allilamines (by inhibition of the squalene epoxidase) or azoles (by inhibition of the cytochrome P-450 enzyme 14α-sterol demethylase). Both mechanisms are common to the fungal pathogen and the host and consequently, drugs that interfere with them affect selectivity towards the pathogen, hence their undesirable side effects. However, the metabolic pathway to the synthesis of sterols also involves differentiated steps in both fungal and mammal organisms, that may be used for blocking growth of the former without affecting the latter. One such step refers to the sterol *C*-methylations catalyzed by the enzyme (*S*)-adenosyl-L-methionine: Δ^{24} - sterol methyl transferase (SMT).[273,291] SMTs are a common occurrence in Nature, though are absent in animal systems, suggesting an interesting alternative in the search for selective antifungals affecting this particular step.[272,273,281] AZA-1 (fig. 4; 22,26-azasterol, 0.1 to 5 μM) inhibited *P. brasiliensis* growth in a dose-dependent fashion, reaching 100% growth arrest at the latter concentration and above,[281] a result that is similar to those previously reported for parasites (*T. cruzi*, *L. donovani*)[273] and fungi (*P. carinii*).[272] AZA-2 (22-piperidin-2-il-pregnan-22(S),3β-diol), instead, was only able to inhibit 60% growth at the highest concentration used in these experiments (10 μM), while AZA-3 (22-piperidin-3-il-pregnan-22(S),3β-diol) was the most powerful drug, since a concentration of 0.5 μM was able to completely

inhibit fungal growth in a fungistatic manner.[281,282] A detailed lipid analysis indicated that on exposure to AZA-1, ergosta-5,7,24(28)-trien-3β-ol (17.1%) and lanosterol (11%) accumulated, while AZA-2 led to an important accumulation of ergosta-5,7,22,24(28)-tetraen-3β-ol (50.5%), a result that suggested a significant inhibition of the $\Delta^{24(28)}$ sterol methyl reductase (SMR), an enzyme that catalyzes the saturation of the $\Delta^{24(28)}$ double bond in the biosynthesis of brassicasterol. With AZA-3, instead, an important accumulation of lanosterol (34.5%) was observed, indicative that a specific blockage of SMT activity was in effect.[273,274] Sterol hydrazones derivatives, such as 20-hydrazone-imidazolin-2-yl-5α-pregnan-3β-ol (H1), 20-hydrazone-pyridin-2-yl-5α-pregnan-3β-ol (H2), 22-hydrazone-imidazolin-2-yl-chol-5-ene-3β-ol (H3) and 22-hydrazone-pyridin-2-yl-chol-5-ene-3β-ol (H4) also show similar inhibitory properties against *P. brasiliensis* SMT [Visbal et al., submitted].

The azasterols 20-piperidin-2-yl-5α-pregnan-3β-20(R)-diol (AZA), and 24(R,S),25-epiminolanosterol (EIL), were tested against 70 clinical isolates of the genus *Candida*.[113] All strains were susceptible to amphotericin B; however, some isolates, mostly *Candida* non-*albicans* such as *C. guilliermondii, Candida zeylanoides*, and *Candida lipolytica*, were fluconazole- and itraconazole-resistant, but susceptible to both AZA and EIL. Reference strain *C. krusei* (ATCC 6258, fluconazole-resistant) was consistently susceptible to AZA, although not to EIL. The fungicidal action of these compounds was more prominent against *Candida* non-*albicans* species than against *C. albicans* isolates. A concentration of 4.0 μg/ml of either compound was enough to kill 100% of *C. lusitanae*, *C. zeylanoides*, and *Candida rugosa*, and 50% of *C. glabrata*. In contrast, this same concentration killed only 4.7% (AZA) and 9.5% (EIL) of *C. albicans* isolates, a result that opens new roads for the eventual use of such drugs as alternative treatments for candidiasis provoked by fluconazole- and itraconazole-resistant *Candida* spp. Treatment with sub-inhibitory concentrations of AZA and EIL induced ultrastructural alterations, such as changes in the cell-wall shape and thickness, a pronounced disconnection between the cell wall and cytoplasm with an electron-lucent zone between them, mitochondrial swelling, and the presence of electron-dense vacuoles. Fluorescence microscopy analyses indicated an accumulation of lipid bodies and alterations in the cell cycle of the yeasts.[113]

The above mentioned research on azasterols and sterol hydrazones has been assisted by the use of methods for the rational design of antifungals[173–175,282] to find the most probable suited molecules for a given action, prior to experiments. QSAR (quantitative structure-activity relationship) investigation was applied to find a correlation between the different physicochemical parameters of a new series of furan-3-carboxamides and their biological activity.[288] QSAR analysis of heterocyclic antifungals was also applied by Duchowicz et al.[82] to 1202 numerical descriptors that encode the various aspects of the topological, geometrical and electronic molecular structure with the aim of achieving the best QSAR relationship between the antifungal potencies against *C. albicans* and the structure of 96 heterocyclic ring derivatives. From the model arisen of such search the authors predicted the biological activity for 60 non-yet measured compounds. Bi- and multilinear PLS (partial least squares) coupled to MIA-QSAR (multivariate image analysis applied to quantitative structure-activity relationship) were used in the prediction of antifungal activities of some benzothiazole derivatives that act as *C. albicans* N-myristoyltransferase (Nmt) inhibitors.[28] Two different regression methods were used: N-PLS, applied to the three-way array, and PLS, applied to the unfolded array. Both models demonstrated excellent predictive ability, with results comparable to those obtained through 3D approaches. In order to compare the results obtained through MIA descriptors with the predictions of a classical 2D QSAR, some representative physicochemical descriptors were calculated and regressed against the experimental pIC50 values through multiple linear regression, demonstrating that MIA-QSAR was superior for this series of compounds.[28]

Contrary to allylamines and azoles, that act by blocking the synthesis of membrane sterols, amphotericin B, the golden standard of antifungals, acts by physically impairing the cell membrane, be it from fungal or mammalian cells. Consequently, while being highly effective as a fungicidal agent, it is also a highly nephrotoxic drug. To alleviate this condition, lipid preparations such as Ambisome are currently available. However, their high cost makes them prohibitive as the drug of choice in poor populations, such as those frequently found in Latin America. Hence, some research is being devoted to the preparation of alternative lipid derivatives of amphotericin B. Amaral et al[6] reported on the preparation and testing of a desoxycholate amphotericin B (D-AMB) sustained delivery system based on poly(lactic-co-glycolic acid) (PLGA) and dimercaptosuccinic acid (DMSA) polymeric blends (Nano-D-AMB) aimed at reducing the number of AMB administrations required to treat mycosis. To this effect, mice were infected with *P. brasiliensis*, Y phase, and treated with Nano-D-AMB (6 mg/kg/every 3 days) or D-AMB (2 mg/kg/daily). Efficacy was comparable in both treatments, although Nano-D-AMB-treated group presented lower loss of body weight and absence of stress sign (piloerection and hypotrichosis) than D-AMB-treated group. No renal (blood urea nitrogen, creatinine) or hepatic (pyruvic and oxalacetic glutamic transaminases) biochemical abnormalities were found. Genotoxic or cytotoxic effects were absent. It was concluded that the D-AMB-coated PLGA-DMSA nanoparticle showed antifungal efficacy, fewer undesirable effects and a favourable extended dosing interval, results that hold promise for improved methods of treatment against systemic fungal infections.

Natural products or chemically derived compounds are being tested for antifungal properties. One preferred target has been the fungal cell wall, a structure absent in mammalian cells, required for the survival of the fungal cells inasmuch as it confers a physical barrier against the internal turgor pressure of the cytoplasm. Argentinean Zacchino and collaborators in several other Latin American countries, among other research groups, have devoted many years to the subject. 4-Aryl- or 4-alkyl-N-arylamine-1-butenes were transformed into 2-substituted 4-methyl-tetrahydroquinolines and 4-pyridyl quinolines that displayed a range of antifungal properties in particular against *Epidermophyton floccosum* and *M. canis*, by way of β-(1-3) glucan-synthase and chitin-synthase inhibition.[159,275] Similar effects were reported for N-phenyl-, N-aryl-, N-phenylalkyl-maleimide and 3,4-dichloromaleimide derivatives[128] when tested against a panel of standardized yeasts and filamentous fungi as well as clinical isolates of *C. albicans*. The activities of N-phenylalkyl-3,4-dichloromaleimide derivatives but not those of N-phenylalkyl-maleimide derivatives showed to be dependent on the length of the alkyl chain, exerting a fungicidal, not fungistatic activity. Some of them possessed strong antifungal activities against all the tested *Candida* strains with MICs between 0.48 and 3.90 μg/ml, values that are similar to those of amphotericin B (0.12–1.56 μg/ml) and in some cases better that those of ketoconazole (0.12–6.25 μg/ml).[243] N-Phenylpropyl-3,4-dichloromaleimide showed the broadest spectrum of action and lower minimal inhibitory concentrations (MIC) in all of the fungi tested. Later on, López et al[129] produced a semisynthetic mixture of compounds by diversification of a natural product extract through the chemical transformation of common chemical functionalities, mainly molecules with a high frequency of carbonyl groups in natural products, into chemical functionalities rarely found in nature, e.g. pyrazoles. The resulting mixture showed antifungal activity against *C. albicans*, whereas the starting extract did not show such activity.

Of the β-1,3-glucan synthase inhibitors so far studied, echinocandins (namely, caspofungin, anidulafungin and micafungin) are the only ones that have made their way into clinics. A recent study[219] indicates that the inhibitory effect of caspofungin on the yeast phase of 5 *P. brasiliensis* strains ranged between 20 and 65%, depending on the strain. The mycelial phase was more susceptible to caspofungin, inhibition varying between 74 and 81%, in agreement with the 3-times higher amount of β-1,3-glucan present in the mycelial cell wall as compared with the yeastlike phase. The variable sensitivity of each strain towards caspofungin, in a given morphological phase, was independent from the different amounts of α- and β-1,3-glucan present in each strain. The drug induced physical changes in the cell walls of both fungal phases, as well as cytoplasmic deterioration. Caspofungin has been reported to have a paradoxical behaviour when used in high concentrations.[249] A recent report by Melo et al[160] points to the paradoxical growth of biofilms of *Candida* sp. clinical isolates (4 *C. albicans*, 6 *C. tropicalis*, 7 *C. parapsilosis*, 8 *C. orthopsilosis*, and 5 *C. metapsilosis*) in the presence of high caspofungin concentrations. With the exception of *C. tropicalis*, all isolates displayed paradoxical growth more frequently when they were grown as biofilms than when grown as planktonic cells.

The characteristics of *C. tropicalis* biofilm formation in vitro were described by Bizerra et al.[29] By an XTT-reduction assay, an increase in metabolic activity was observed up to 24 h of biofilm formation, and this activity showed a linear relationship with sessile cell density. Mature biofilms consisted of a dense network of yeast cells and filamentous forms of *C. tropicalis*. Increased resistance of sessile cells against fluconazole and amphotericin B was also demonstrated. Real-time reverse transcription-PCR quantification showed that sessile cells overexpressed ERG11 (coding for lanosterol 14 alpha-demethylase) and MDR1 (coding for an efflux protein belonging to the major facilitator superfamily). These mechanisms may contribute to the fluconazole resistance of the *C. tropicalis* biofilm. *C. albicans* secretory aspartyl proteinase, a virulence factor, has been reported to be enhanced in biofilms.[161,172]

Multiple resistance mechanisms among *A. fumigatus* mutants with high-level resistance to itraconazole were found to reside in point at Gly54 (G54E, -K, or -R) in the azole target gene CYP51A,[176] in agreement with information from other sources.[283] Additionally, two genes, AfuMDR3 and AfuMDR4, showed prominent changes in expression levels in many highly resistant mutants. Analysis of the deduced amino acid sequence encoded by AfuMDR3 revealed high similarity to major facilitator superfamily transporters, while AfuMDR4 was a typical member of the ATP-binding cassette superfamily. By real-time quantitative PCR it was shown that overexpression of one or both of these newly identified drug efflux pump genes of *A. fumigatus* and/or selection of drug target site mutations are linked to high-level itraconazole resistance and are mechanistic considerations for the emergence of clinical resistance to itraconazole. Of the five sequential *C. neoformans* isolates recovered from an AIDS patient with recurrent meningitis, four isolates were fluconazole susceptible, while the fifth isolate developed fluconazole resistance, due to a point mutation (G484S) in the 14-alpha lanosterol demethylase gene (ERG11).[215]

To provide insights in drug resistance, transporter in *P. brasiliensis* were deduced by data mining in its transcriptome.[63] Twenty two groups with good similarity with other fungal ATP binding cassette transporters, and four *P. brasiliensis* sequence tags that probably code for major facilitator superfamily proteins were found, among them, homologs to *C. albicans* CDR1, CDR2, and MDR1, *S. cerevisiae* PDR5 and *Aspergillus* AtrF genes, all of them related to azole resistance. Also in *T. rubrum*, a gene encoding an ABC transporter, TruMDR1, was cloned. The open reading frame of TruMDR1 was 4838 bp long and the deduced amino acid sequence showed high homology with ABC transporters involved in drug efflux of other fungi. An increase in expression level was observed when the fungus was exposed to ethidium bromide, ketoconazole, cycloheximide, fluconazole, griseofulvin, imazalil and itraconazole, suggesting the participation of this gene in drug efflux in this dermatophyte.[53]

Conclusions

This account of highlighted experimental medical mycological research in Latin America in the initial years of the 21st Century provides us with the notion that despite chronic difficulties in our region, there are groups working hard to advance local research at international levels of quality, frequently within programs of international cooperation. The potentiality exists to keep and improve such level of excellent performance, and to provide more opportunities for the training of young researchers. For this to be achieved, good will, strong governmental policies and funds are required in order that society, as a whole, profits from advances in scientific knowledge.

EB acknowledges the finantial support of FAPESP and CNPq 304630/2009-8 through grants No. 06-60091-6 and 304630/2009-8, respectively.

Conflicts of interest

There are no conflicts of interest in either author.

References

1. Albuquerque PC, Nakayasu ES, Rodrigues ML, Frases S, Casadevall A, Zancope-Oliveira RM, et al. Vesicular transport in *Histoplasma capsulatum*: an effective mechanism for trans-cell wall transfer of proteins and lipids in ascomycetes. Cell Microbiol. 2008;10:1695–710.
2. Almeida AJ, Cunha C, Carmona JA, Sampaio-Marques B, Carvalho A, Malavazi I, et al. Cdc42p controls yeast-cell shape and virulence of *Paracoccidioides brasiliensis*. Fungal Genet Biol. 2009;46:919–26.
3. Almeida AJ, Matute DR, Carmona JA, Martins M, Torres I, McEwen JG, et al. Genome size and ploidy of *Paracoccidioides brasiliensis* reveals a haploid DNA content: flow cytometry and GP43 sequence analysis. Fungal Genet Biol. 2007;44:25–31.
4. Almeida GM, Andrade RM, Bento CA. The capsular polysaccharides of *Cryptococcus neoformans* activate normal CD4(+) T cells in a dominant Th2 pattern. J Immunol. 2001;167:5845–51.
5. Almeida-Paes R, Pimenta MA, Monteiro PC, Nosanchuk JD, Zancopé-Oliveira RM. Immunoglobulins G, M, and A against *Sporothrix schenckii* exoantigens in patients with sporotrichosis before and during treatment with itraconazole. Clin Vaccine Immunol. 2007;14:1149–57.
6. Amaral AC, Bocca AL, Ribeiro AM, Nunes J, Peixoto DL, Simioni AR, et al. Amphotericin B in poly(lactic-co-glycolic acid) (PLGA) and dimercaptosuccinic acid (DMSA) nanoparticles against PCM. J Antimicrob Chemother. 2009;63:526–33.
7. Apitz-Castro R, Escalante J, Vargas R, Jain MK. Ajoene, the antiplatelet principle of garlic, synergistically potentiates the antiaggregatory action of prostacyclin, forskolin, indomethacin and dypiridamole on human platelets. Thromb Res. 1986;42:303–11.
8. Aquino-Piñero E, Rodríguez-del Valle N. Characterization of a protein kinase C gene in *Sporothrix schenckii* and its expression during the yeast-to-mycelium transition. Med Mycol. 2002;40:185–99.
9. Arroyo-Flores BL, Calvo-Méndez C, Flores-Carreón A, López-Romero E. Biosynthesis of glycoproteins in the pathogenic fungus *Candida albicans*: activation of dolichol phosphate mannose synthase by cAMP-mediated protein phosphorylation. FEMS Immunol Med Microbiol. 2005;45:429–34.
10. Arroyo-Flores BL, Calvo-Méndez C, Flores-Carreón A, López-Romero E. Partial purification and characterization of a mannosyl transferase involved in O -linked mannosylation of glycoproteins in *Candida albicans*. Antonie Van Leeuwenhoek. 2004;85:199–207.
11. Arroyo-Flores BL, Rodríguez-Bonilla J, Villagómez-Castro JC, Calvo-Méndez C, Flores-Carreón A, López-Romero E. Biosynthesis of glycoproteins in *Candida albicans*: activity of mannosyl and glucosyl transferases. Fungal Genet Biol. 2000;30:127–33.
12. Arruda C, Franco MF, Kashino SS, Nascimento FR, Fazioli R, dos A, Vaz CA, et al. Interleukin-12 protects mice against disseminated infection caused by *Paracoccidioides brasiliensis* but enhances pulmonary inflammation. Clin Immunol. 2002;103:185–95.
13. Bailão AM, Schrank A, Borges CL, Dutra V, Walquíria I, Molinari-Madlum EE, et al. Differential gene expression by *Paracoccidioides brasiliensis* in host interaction conditions: representational difference analysis identifies can-

didate genes associated with fungal pathogenesis. Microbes Infect. 2006;8: 2686–97.
14. Baires-Varguez L, Cruz-García A, Villa-Tanaka L, Sánchez-García S, Gaitán-Cepeda LA, Sánchez-Vargas LO, et al. Comparison of a randomly amplified polymorphic DNA (RAPD) analysis and ATB ID 32C system for identification of clinical isolates of different *Candida* species. Rev Iberoam Micol. 2007;24:148–51.
15. Barreto L, Sorais F, Salazar V, San-Blas G, Niño-Vega GA. Expression of *Paracoccidioides brasiliensis* CHS3 in a *Saccharomyces cerevisiae* chs3 null mutant demonstrates its functionality as a chitin synthase gene. Yeast. 2010;27:293–300.
16. Barros TF, Puccia R. Cloning and characterization of a LON gene homologue from the human pathogen *Paracoccidioides brasiliensis*. Yeast. 2001;18:981–8.
17. Barrozo LV, Mendes RP, Marques SA, Benard G, Silva ME, Bagagli E. Climate and acute/subacute PCM in a hyper-endemic area in Brazil. Int J Epidemiol. 2009;38:1642–9.
18. Bastos KP, Bailão AM, Borges CL, Faria FP, Felipe MS, Silva MG, et al. The transcriptome analysis of early morphogenesis in *Paracoccidioides brasiliensis* mycelium reveals novel and induced genes potentially associated to the dimorphic process. BMC Microbiol. 2007;7:29.
19. Batista WL, Barros TF, Goldman GH, Morais FV, Puccia R. Identification of transcription elements in the 5' intergenic region shared by LON and MDJ1 heat shock genes from the human pathogen *Paracoccidioides brasiliensis*. Evaluation of gene expression. Fungal Genet Biol. 2007;44:347–56.
20. Bautista-Muñoz C, Boldo XM, Villa-Tanaca L, Hernández-Rodríguez C. Identification of *Candida* spp. by randomly amplified polymorphic DNA analysis and differentiation between *Candida albicans* and *Candida dubliniensis* by direct PCR methods. J Clin Microbiol. 2003;41:414–20.
21. Bautista-Muñoz C, Hernández-Rodríguez C, Villa-Tanaca L. Analysis and expression of STE13ca gene encoding a putative X-prolyl dipeptidyl aminopeptidase from *Candida albicans*. FEMS Immunol Med Microbiol. 2005;45:459–69.
22. Bernardo SM, Khalique Z, Kot J, Jones JK, Lee SA. *Candida albicans* VPS1 contributes to protease secretion, filamentation, and biofilm formation. Fungal Genet Biol. 2008;45:861–77.
23. Bertini S, Colombo AL, Takahashi HK, Straus AH. Expression of antibodies directed to *Paracoccidioides brasiliensis* glycosphingolipids during the course of PCM treatment. Clin Vaccine Immunol. 2007;14:150–6.
24. Berzaghi R, Colombo AL, Machado AM, de Camargo ZP. New approach for diagnosis of candidemia based on detection of a 65-kilodalton antigen. Clin Vaccine Immunol. 2009;16:1538–45.
25. Bialek R, Ibricevic A, Aepinus C, Najvar LK, Fothergill AW, Knobloch J, et al. Detection of *Paracoccidioides brasiliensis* in tissue samples by a nested PCR assay. J Clin Microbiol. 2000;38:2940–2.
26. Bialek R, Kern J, Herrmann T, Tijerina R, Ceceñas L, Reischl U, et al. PCR assays for identification of *Coccidioides posadasii* based on the nucleotide sequence of the antigen 2/proline-rich antigen. J Clin Microbiol. 2004;42:778–83.
27. Biselli PJ, Juvenale M, Mendes-Giannini MJ, Duarte AJ, Benardi G. IgE antibody response to the main antigenic component of *Paracoccidioides brasiliensis* in patients with PCM. Med Mycol. 2001;39:475–8.
28. Bitencourt M, Freitas MP. Bi- and multilinear PLS coupled to MIA-QSAR in the prediction of antifungal activities of some benzothiazole derivatives. Med Chem. 2009;5:79–86.
29. Bizerra FC, Nakamura CV, de Poersch C, Estivalet Svidzinski TI, Borsato Quesada RM, Goldenberg S, et al. Characteristics of biofilm formation by *Candida tropicalis* and antifungal resistance. FEMS Yeast Res. 2008;8:442–50.
30. Bocca AL, Brito PP, Figueiredo F, Tosta CE. Inhibition of nitric oxide production by macrophages in chromoblastomycosis: a role for *Fonsecaea pedrosoi* melanin. Mycopathologia. 2006;161:195–203.
31. Bordon AP, Dias-Melicio LA, Acorci MJ, Calvi SA, Serrão Peraçoli MT, Victoriano de Campos Soares AM. Prostaglandin E2 inhibits *Paracoccidioides brasiliensis* killing by human monocytes. Microbes Infect. 2007;9:744–7.
32. Bracca A, Tosello ME, Girardini JE, Amigot SL, Gómez C, Serra E. Molecular detection of *Histoplasma capsulatum* var. *capsulatum* in human clinical samples. J Clin Microbiol. 2003;41:1753–5.
33. Braga CJ, Rittner GM, Muñoz Henao JE, Teixeira AF, Massis LM, Sbrogio-Almeida ME, et al. *Paracoccidioides brasiliensis* vaccine formulations based on the gp43-derived P10 sequence and the *Salmonella enterica* FliC flagellin. Infect Immun. 2009;77:1700–7.
34. Brito Gamboa A, Mendoza M, Fernández A, Díaz E. Detection of *Candida dubliniensis* in patients with candidiasis in Caracas. Venezuela Rev Iberoam Micol. 2006;23:81–4.
35. Buissa-Filho R, Puccia R, Marques AF, Pinto FA, Muñoz JE, Nosanchuk JD, et al. The monoclonal antibody against the major diagnostic antigen of *Paracoccidioides brasiliensis* mediates immune protection in infected BALB/c mice challenged intratracheally with the fungus. Infect Immun. 2008;76:3321–8.
36. Cacere CR, Mendes-Giannini MJ, Fontes CJ, Kono A, Duarte AJ, Benard G. Altered expression of the costimulatory molecules CD80, CD86, CD152, PD-1 and ICOS on T-cells from paracoccidioidomycosis patients: lack of correlation with T-cell hyporesponsiveness. Clin Immunol. 2008;129:341–9.
37. Calcagno AM, Niño-Vega G, San-Blas F, San-Blas G. Geographic discrimination of *Paracoccidioides brasiliensis* strains by randomly amplified polymorphic DNA analysis. J Clin Microbiol. 1998;36:1733–6.
38. Calich VL, Pina A, Felonato M, Bernardino S, Costa TA, Loures FV. Toll-like receptors and fungal infections: the role of TLR2, TLR4 and MyD88 in PCM. FEMS Immunol Med Microbiol. 2008;53:1–7.
39. Calvi SA, Peraçoli MTS, Mendes RP, Marcondes-Machado J, Fecchio D, Marques SA, et al. Effect of cytokines on the in vitro fungicidal activity of monocytes from paracoccidioidomycosis patients. Microbes Infect. 2003;5:107–13.
40. Campos CB, Di Benedette JP, Morais FV, Ovalle R, Nobrega MP. Evidence for the role of calcineurin in morphogenesis and calcium homeostasis during mycelium-to-yeast dimorphism of *Paracoccidioides brasiliensis*. Eukaryot Cell. 2008;7:1856–64.
41. Campos MR, Russo M, Gomes E, Almeida SR. Stimulation, inhibition and death of macrophages infected with *Trichophyton rubrum*. Microbes Infect. 2006;8:372–9.
42. Canteros CE, Zuiani MF, Ritacco V, Perrotta DE, Reyes-Montes MR, Granados J, et al. Electrophoresis karyotype and chromosome-length polymorphism of *Histoplasma capsulatum* clinical isolates from Latin America. FEMS Immunol Med Microbiol. 2005;45:423–8.
43. Carlos IZ, Sgarbi DB, Santos GC, Placeres MC. *Sporothrix schenckii* lipid inhibits macrophage phagocytosis: involvement of nitric oxide and tumour necrosis factor-alpha. Scand J Immunol. 2003;57:214–20.
44. Carrero LL, Niño-Vega G, Teixeira MM, Carvalho MJ, Soares CM, Pereira M, et al. New *Paracoccidioides brasiliensis* isolate reveals unexpected genomic variability in this human pathogen. Fungal Genet Biol. 2008;45:605–12.
45. Carvalho KC, Ganiko L, Batista WL, Morais FV, Marques ER, Goldman GH, et al. Virulence of *Paracoccidioides brasiliensis* and gp43 expression in isolates bearing known *PbGP43* genotype. Microbes Infect. 2005;7:55–65.
46. Casadevall A, Pirofski LA. Antibody-mediated protection through cross-reactivity introduces a fungal heresy into immunological dogma. Infect Immun. 2007;75:5074–8.
47. Cassola A, Parrot M, Silberstein S, Magee BB, Passeron S, Giasson L, et al. *Candida albicans* lacking the gene encoding the regulatory subunit of protein kinase A displays a defect in hyphal formation and an altered localization of the catalytic subunit. Eukaryot Cell. 2004;3:190–9.
48. Cassone A. Fungal vaccines: real progress from real challenges. Lancet Infect Dis. 2008;8:114–24.
49. Castañón-Olivares LR, Güereña-Elizalde D, González-Martínez MR, Licea-Navarro AF, González-González GM, Aroch-Calderón A. Molecular identification of *Coccidioides* isolates from Mexican patients. Ann N Y Acad Sci. 2007;1111:326–35.
50. Castro N, da S, de Castro KP, Orlandi I, Feitosa L, dos S, Rosa e Silva LK, Vainstein MH, et al. Characterization and functional analysis of the beta-1,3-glucanosyltransferase 3 of the human pathogenic fungus *Paracoccidioides brasiliensis*. FEMS Yeast Res. 2009;9:103–14.
51. Cavalheiro RA, Fortes F, Borecký J, Faustinoni VC, Schreiber AZ, Vercesi AE. Respiration, oxidative phosphorylation, and uncoupling protein in *Candida albicans*. Braz J Med Biol Res. 2004;37:1455–61.
52. Cervantes-Chávez JA, Ruiz-Herrera J. The regulatory subunit of protein kinase A promotes hyphal growth and plays an essential role in *Yarrowia lipolytica*. FEMS Yeast Res. 2007;7:929–40.
53. Cervelatti EP, Fachin AL, Ferreira-Nozawa MS, Martinez-Rossi NM. Molecular cloning and characterization of a novel ABC transporter gene in the human pathogen *Trichophyton rubrum*. Med Mycol. 2006;44:141–7.
54. Chagas RF, Bailão AM, Pereira M, Winters MS, Smullian AG, Deepe Jr GS, et al. The catalases of *Paracoccidioides brasiliensis* are differentially regulated: protein activity and transcript analysis. Fungal Genet Biol. 2008;45: 1470–8.
55. Chiapello LS, Baronetti JL, Aoki MP, Gea S, Rubinstein H, Masih DT. Immunosuppression, interleukin-10 synthesis and apoptosis are induced in rats inoculated with *Cryptococcus neoformans* glucuronoxylomannan. Immunology. 2004;113:392–400.
56. Chiapello LS, Baronetti JL, Garro AP, Spesso MF, Masih DT. *Cryptococcus neoformans* glucuronoxylomannan induces macrophage apoptosis mediated by nitric oxide in a caspase-independent pathway. Int Immunol. 2008;20:1527–41.
57. Chiarella AP, Arruda C, Pina A, Costa TA, Ferreira RC, Calich VL. The relative importance of $CD4^+$ and $CD8^+$T cells in immunity to pulmonary PCM. Microbes Infect. 2007;9:1078–88.
58. Cloutier M, Castilla R, Bolduc N, Zelada A, Martineau P, Bouillon M, et al. The two isoforms of the cAMP-dependent protein kinase catalytic subunit are involved in the control of dimorphism in the human fungal pathogen *Candida albicans*. Fungal Genet Biol. 2003;38:133–41.
59. Collopy-Junior I, Kneipp LF, da Silva FC, Rodrigues ML, Alviano CS, Meyer-Fernandes JR. Characterization of an ecto-ATPase activity in *Fonsecaea pedrosoi*. Arch Microbiol. 2006;185:355–62.
60. Colombo AL, Nucci M, Park BJ, Nouér SA, Arthington-Skaggs B, da Matta DA, et al., Brazilian Network Candidemia Study. Epidemiology of candidemia in Brazil: a nationwide sentinel surveillance of candidemia in eleven medical centers. J Clin Microbiol. 2006;44:2816–23.
61. Corbellini VA, Scroferneker ML, Carissimi M, Santolin LD. Delayed-type hypersensitivity response to crude and fractionated antigens from *Fonsecaea pedrosoi* CMMI 1 grown in different culture media. Mycopathologia. 2006;162:51–5.
62. Corvino CL, Mamoni RL, Fagundes GZ, Blotta MH. Serum interleukin-18 and soluble tumour necrosis factor receptor 2 are associated with disease severity in patients with paracoccidioidomycosis. Clin Exp Immunol. 2007;147:483–90.
63. Costa C, da S, Albuquerque FC, Andrade RV, Oliveira GC, Almeida MF, Brigido Mde M, et al. Transporters in the *Paracoccidioides brasiliensis* transcriptome: insights on drug resistance. Genet Mol Res. 2005;4:390–408.

64. Costa DL, Dias-Melicio LA, Acorci MJ, Bordon AP, Tavian EG, Peraçoli MT, et al. Effect of interleukin-10 on the *Paracoccidioides brasiliensis* killing by gamma-interferon activated human neutrophils. Microbiol Immunol. 2007;51:73–80.
65. Costa M, Borges CL, Bailão AM, Meirelles GV, Mendonça YA, Dantas SF, et al. Transcriptome profiling of *Paracoccidioides brasiliensis* yeast-phase cells recovered from infected mice brings new insights into fungal response upon host interaction. Microbiology. 2007;153:4194–207.
66. Cuéllar-Cruz M, Briones-Martin-del-Campo M, Cañas-Villamar I, Montalvo-Arredondo J, Riego-Ruiz L, Castaño I, et al. High resistance to oxidative stress in the fungal pathogen *Candida glabrata* is mediated by a single catalase, Cta1p, and is controlled by the transcription factors Yap1p, Skn7p, Msn2p, and Msn4p. Eukaryot Cell. 2008;7:814–25.
67. Cuenca-Estrella M, Rodríguez-Tudela JL, Córdoba S, Melhem MC, Szeszs MW, Castañeda E, et al. Red regional de laboratorios para la vigilancia de las infecciones fúngicas invasoras y susceptibilidad a los antifúngicos [Regional laboratory network for surveillance of invasive fungal infections and antifungal susceptibility in Latin America]. Rev Panam Salud Pública. 2008;23:129–34.
68. Da Rosa D, Gezuele E, Calegari L, Goñi F. Excretion-secretion products and proteases from live *Sporothrix schenckii* yeast phase: immunological detection and cleavage of human IgG. Rev Inst Med Trop Sao Paulo. 2009;51:1–7.
69. Da Silva JP, da Silva MB, Salgado UI, Diniz JA, Rozental S, Salgado CG. Phagocytosis of *Fonsecaea pedrosoi* conidia, but not sclerotic cells caused by Langerhans cells, inhibits CD40 and B7-2 expression. FEMS Immunol Med Microbiol. 2007;50:104–11.
70. Dantas AS, Andrade RV, de Carvalho MJ, Felipe MS, Campos EG. Oxidative stress response in *Paracoccidioides brasiliensis*: assessing catalase and cytochrome c peroxidase. Mycol Res. 2008;112:747–56.
71. De Aguiar Cordeiro R, Nogueira Brilhante RS, Gadelha Rocha MF, Araújo Moura FE, Pires de Camargo Z, Costa Sidrim JJ. Rapid diagnosis of coccidioidomycosis by nested PCR assay of sputum. Clin Microbiol Infect. 2007;13:449–51.
72. De Aguirre L, Hurst SF, Choi JS, Shin JH, Hinrikson HP, Morrison CJ. Rapid differentiation of *Aspergillus* species from other medically important opportunistic molds and yeasts by PCR-enzyme immunoassay. J Clin Microbiol. 2004;42:3495–504.
73. De Andrade TS, Cury AE, de Castro LG, Hirata MH, Hirata RD. Rapid identification of *Fonsecaea* by duplex polymerase chain reaction in isolates from patients with chromoblastomycosis. Diagn Microbiol Infect Dis. 2007;57:267–72.
74. De Gouvêa PF, Soriani FM, Malavazi I, Savoldi M, Goldman MH, Loss O, et al. Functional characterization of the *Aspergillus fumigatus* PHO80 homologue. Fungal Genet Biol. 2008;45:1135–46.
75. De Jesús-Berríos M, Rodríguez-del Valle N. Expression of a Pho85 cyclin-dependent kinase is repressed during the dimorphic transition in *Sporothrix schenckii*. Fungal Genet Biol. 2002;37:39–48.
76. Delgado N, Rodríguez-del Valle N. Presence of a pertussis toxin-sensitive G protein alpha subunit in *Sporothrix schenckii*. Med Mycol. 2000;38:109–21.
77. Díaz MR, Boekhout T, Kiesling T, Fell JW. Comparative analysis of the intergenic spacer regions and population structure of the species complex of the pathogenic yeast *Cryptococcus neoformans*. FEMS Yeast Res. 2005;5:1129–40.
78. Dolande Franco ME, Reviákina V, Panizo MM, Macero C, Moreno X, Calvo A, et al. Distribución y sensibilidad a los antifúngicos de aislamientos clínicos de *Candida* en seis centros de salud del area metropolitana de Caracas, Venezuela (años 2003-2005). Rev Iberoam Micol. 2008;25:17–21.
79. Dos Santos AL, de Carvalho IM, da Silva BA, Portela MB, Alviano CS, de Araújo Soares RM. Secretion of serine peptidase by a clinical strain of *Candida albicans*: influence of growth conditions and cleavage of human serum proteins and extracellular matrix components. FEMS Immunol Med Microbiol. 2006;46:209–20.
80. Dos Santos DF, Nicolete R, de Souza PR, Bitencourt Cda S, dos Santos Jr RR, Bonato VL, et al. Characterization and in vitro activities of cell-free antigens from *Histoplasma capsulatum*-loaded biodegradable microspheres. Eur J Pharm Sci. 2009;38:548–55.
81. Duarte-Escalante E, Zenteno E, Taylor ML. Interaction of *Histoplasma capsulatum* yeasts with galactosylated surface molecules of murine macrophages. Arch Med Res. 2003;34:176–83.
82. Duchowicz PR, Vitale MG, Castro EA, Fernández M, Caballero J. QSAR analysis for heterocyclic antifungals. Bioorg Med Chem. 2007;15:2680–9.
83. Dunn MF, Ramírez-Trujillo JA, Hernández-Lucas I. Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis. Microbiology. 2009;155:3166–75.
84. Escandón P, Sánchez A, Martínez M, Meyer W, Castañeda E. Molecular epidemiology of clinical and environmental isolates of the *Cryptococcus neoformans* species complex reveals a high genetic diversity and the presence of the molecular type VGII mating type a in Colombia. FEMS Yeast Res. 2006;6:625–35.
85. Feitosa L, dos S, Cisalpino PS, dos Santos MR, Mortara RA, Barros TF, Morais FV, et al. Chromosomal polymorphism, syntenic relationships, and ploidy in the pathogenic fungus *Paracoccidioides brasiliensis*. Fungal Genet Biol. 2003;39:60–9.
86. Felipe MS, Andrade RV, Petrofeza SS, Maranhão AQ, Torres FA, Albuquerque P, et al. Transcriptome characterization of the dimorphic and pathogenic fungus *Paracoccidioides brasiliensis* by EST analysis. Yeast. 2003;20:263–71.
87. Fernandes KS, Neto EH, Brito MM, Silva JS, Cunha FQ, Barja-Fidalgo C. Detrimental role of endogenous nitric oxide in host defence against *Sporothrix schenckii*. Immunology. 2008;123:469–79.
88. Fernández Murray P, Biscoglio MJ, Passeron S. Purification and characterization of *Candida albicans* 20S proteasome: identification of four proteasomal subunits. Arch Biochem Biophys. 2000;375:211–9.
89. Fernández Murray P, Pardo PS, Zelada AM, Passeron S. *In vivo* and *in vitro* phosphorylation of *Candida albicans* 20S proteasome. Arch Biochem Biophys. 2002;404:116–25.
90. Ferreira KS, Bastos KR, Russo M, Almeida SR. Interaction between *Paracoccidioides brasiliensis* and pulmonary dendritic cells induces interleukin-10 production and toll-like receptor-2 expression: possible mechanisms of susceptibility. J Infect Dis. 2007;196:1108–15.
91. Figueiredo CC, Deccache PM, Lopes-Bezerra LM, Morandi V. TGF-β1 induces transendothelial migration of the pathogenic fungus *Sporothrix schenckii* by a paracellular route involving extracellular matrix proteins. Microbiology. 2007;153:2910–21.
92. Findley K, Rodríguez-Carres M, Metin B, Kroiss J, Fonseca A, Vilgalys R, et al. Phylogeny and phenotypic characterization of pathogenic *Cryptococcus* species and closely related saprobic taxa in the Tremellales. Eukaryot Cell. 2009;8:353–61.
93. Fisher MC, Koenig GL, White TJ, San-Blas G, Negroni R, Alvarez IG, et al. Biogeographic range expansion into South America by *Coccidioides immitis* mirrors New World patterns of human migration. Proc Natl Acad Sci USA. 2001;98:4558–62.
94. Fisher MC, Koenig GL, White TJ, Taylor JW. Molecular and phenotypic description of *Coccidioides posadasii* sp. nov., previously recognized as the non-Californian population of *Coccidioides immitis*. Mycologia. 2002;94:73–84.
95. Fonseca FL, Frases S, Casadevall A, Fischman-Gompertz O, Nimrichter L, Rodrigues ML. Structural and functional properties of the *Trichosporon asahii* glucuronoxylomannan. Fungal Genet Biol. 2009;46:496–505.
96. Fonseca FL, Nimrichter L, Cordero RJ, Frases S, Rodrigues J, Goldman DL, et al. Role for chitin and chitooligomers in the capsular architecture of *Cryptococcus neoformans*. Eukaryot Cell. 2009;8:1543–53.
97. Fornari MC, Bava AJ, Guereño MT, Berardi VE, Silaf MR, Negroni R, et al. High serum interleukin-10 and tumor necrosis factor alpha levels in chronic paracoccidioidomycosis. Clin Diagn Lab Immunol. 2001;8:1036–8.
98. García AM, Hernández O, Aristizábal BH, De Souza Bernardes LA, Puccia R, Naranjo TW, et al. Gene expression analysis of *Paracoccidioides brasiliensis* transition from conidium to yeast cell. Med Mycol. 2010;48:147–54.
99. Gasparoto TH, Gaziri LC, Burger E, de Almeida RS, Felipe I. Apoptosis of phagocytic cells induced by *Candida albicans* and production of IL-10. FEMS Immunol Med Microbiol. 2004;42:219–24.
100. Giacometti R, Kronberg F, Biondi RM, Passeron S. Catalytic isoforms Tpk1 and Tpk2 of *Candida albicans* PKA have non-redundant roles in stress response and glycogen storage. Yeast. 2009;26:273–85.
101. Giacometti R, Souto G, Silberstein S, Giasson L, Cantore ML, Passeron S. Expression levels and subcellular localization of Bcy1p in *Candida albicans* mutant strains devoid of one BCY1 allele results in a defective morphogenetic behavior. Biochim Biophys Acta. 2006;1763:64–72.
102. Goldman GH, dos Reis Marques E, Duarte Ribeiro DC, de Souza Bernardes LA, Quiapin AC, Vitorelli PM, et al. Expressed sequence tag analysis of the human pathogen *Paracoccidioides brasiliensis* yeast phase: identification of putative homologues of *Candida albicans* virulence and pathogenicity genes. Eukaryot Cell. 2003;2:34–48.
103. Gomes GM, Cisalpino PS, Taborda CP, Camargo ZP. PCR for diagnosis of paracoccidioidomycosis. J Clin Microbiol. 2000;38:3478–80.
104. Gonçalves SS, Amorim CS, Nucci M, Padovan AC, Briones MR, Melo AS, et al. Prevalence rates and antifungal susceptibility profiles of *Candida parapsilosis* species complex: results from a nationwide surveillance of candidaemia in Brazil. Clin Microbiol Infect. 2010;16:885–7.
105. González A, de Gregori W, Velez D, Restrepo A, Cano LE. Nitric oxide participation in the fungicidal mechanism of gamma interferon-activated murine macrophages against *Paracoccidioides brasiliensis* conidia. Infect Immun. 2000;68:2546–52.
106. González A, Gómez BL, Muñoz C, Aristizábal BH, Restrepo A, Hamilton AJ, et al. Involvement of extracellular matrix proteins in the course of experimental paracoccidioidomycosis. FEMS Immunol Med Microbiol. 2008;53:114–25.
107. González A, Sahaza JH, Ortiz BL, Restrepo A, Cano LE. Production of pro-inflammatory cytokines during the early stages of experimental *Paracoccidioides brasiliensis* infection. Med Mycol. 2003;41:391–9.
108. González A, Yáñez A, Gozalbo D, Gil ML. MyD88 is dispensable for resistance to *Paracoccidioides brasiliensis* in a murine model of blood-borne disseminated infection. FEMS Immunol Med Microbiol. 2008;54:365–74.
109. González GM, Elizondo M, Ayala J. Trends in species distribution and susceptibility of bloodstream isolates of *Candida* collected in Monterrey, Mexico, to seven antifungal agents: results of a 3-year (2004 to 2007) surveillance study. J Clin Microbiol. 2008;46:2902–5.
110. Gow NAR, Netea MG, Munro CA, Ferwerda G, Bates S, Mora-Montes HM, et al. Immune recognition of *Candida albicans* β-glucan by dectin-1. J Infect Dis. 2007;196:1565–71.
111. Guedes HL, Guimaraes AJ, Muniz M, de M, Pizzini CV, Hamilton AJ, Peralta JM, et al. PCR assay for identification of *Histoplasma capsulatum* based on the nucleotide sequence of the M antigen. J Clin Microbiol. 2003;41:535–9.
112. Hebeler-Barbosa F, Montenegro MR, Bagagli E. Virulence profiles of ten *Paracoccidioides brasiliensis* isolates obtained from armadillos (*Dasypus novemcinctus*). Med Mycol. 2003;41:89–96.

113. Ishida K, Rodrigues JC, Ribeiro MD, Vila TV, de Souza W, Urbina JA, et al. Growth inhibition and ultrastructural alterations induced by Delta 24(25)-sterol methyltransferase inhibitors in *Candida* spp. isolates, including non-*albicans* organisms. BMC Microbiol. 2009;9:74.
114. Jewtuchowicz VM, Mujica MT, Brusca MI, Sordelli N, Malzone MC, Pola SJ, et al. Phenotypic and genotypic identification of *Candida dubliniensis* from subgingival sites in immunocompetent subjects in Argentina. Oral Microbiol Immunol. 2008;23:505–9.
115. Jiménez M, del P, Restrepo A, Radzioch D, Cano LE, García LF. Importance of complement 3 and mannose receptors in phagocytosis of *Paracoccidioides brasiliensis* conidia by Nramp1 congenic macrophages lines. FEMS Immunol Med Microbiol. 2006;47:56–66.
116. Junior IC, Rodrigues ML, Alviano CS, Travassos LR, Meyer-Fernandes JR. Characterization of an ecto-ATPase activity in *Cryptococcus neoformans*. FEMS Yeast Res. 2005;5:899–907.
117. Kashino SS, Fazioli RA, Cafalli-Favati C, Meloni-Bruneri LH, Vaz CA, Burger E, et al. Resistance to *Paracoccidioides brasiliensis* infection is linked to a preferential Th1 immune response, whereas susceptibility is associated with absence of IFN-gamma production. J Interferon Cytokine Res. 2000;20: 89–97.
118. Kneipp LF, Rodrigues ML, Holandino C, Esteves FF, Souto-Padrón T, Alviano CS, et al. Ectophosphatase activity in conidial forms of *Fonsecaea pedrosoi* is modulated by exogenous phosphate and influences fungal adhesion to mammalian cells. Microbiology. 2004;150:3355–62.
119. Kwon-Chung KJ, Varma A. Do major species concepts support one, two or more species within *Cryptococcus neoformans*? FEMS Yeast Res. 2006;6:574–87.
120. Levery SB, Momany M, Lindsey R, Toledo MS, Shayman JA, Fuller M, et al. Disruption of the glucosylceramide biosynthetic pathway in *Aspergillus nidulans* and *Aspergillus fumigatus* by inhibitors of UDP-Glc:ceramide glucosyltransferase strongly affects spore germination, cell cycle, and hyphal growth. FEBS Lett. 2002;525:59–64.
121. Lima OC, Bouchara JP, Renier G, Marot-Leblond A, Chabasse D, Lopes-Bezerra LM. Immunofluorescence and flow cytometry analysis of fibronectin and laminin binding to *Sporothrix schenckii* yeast cells and conidia. Microb Pathog. 2004;37:131–40.
122. Lima OC, Figueiredo CC, Previato JO, Mendonça-Previato L, Morandi V, Lopes Bezerra LM. Involvement of fungal cell wall components in adhesion of *Sporothrix schenckii* to human fibronectin. Infect Immun. 2001;69:6874–80.
123. Lima-Neto RG, Beltrão EI, Oliveira PC, Neves RP. Adherence of *Candida albicans* and *Candida parapsilosis* to epithelial cells correlates with fungal cell surface carbohydrates. Mycoses. 2009;54:23–9.
124. Livonesi MC, Rossi MA, de Souto JT, Campanelli AP, de Sousa RL, Maffei CM, et al. Inducible nitric oxide synthase-deficient mice show exacerbated inflammatory process and high production of both Th1 and Th2 cytokines during paracoccidioidomycosis. Microbes Infect. 2009;11:123–32.
125. Livonesi MC, Souto JT, Campanelli AP, Maffei CM, Martínez R, Rossi MA, et al. Deficiency of IL-12p40 subunit determines severe paracoccidioidomycosis in mice. Med Mycol. 2008;46:637–46.
126. Loaiza-Loeza S, Parra-Ortega B, Cancino-Díaz JC, Illades-Aguiar B, Hernández-Rodríguez CH, Villa-Tanaca L. Differential expression of *Candida dubliniensis*-secreted aspartyl proteinase genes (CdSAP1-4) under different physiological conditions and during infection of a keratinocyte culture. FEMS Immunol Med Microbiol. 2009;56:212–22.
127. Lopes BG, Santos AL, Bezerra Cde C, Wanke B, Dos Santos Lazéra M, Nishikawa MM, et al. A 25-kDa serine peptidase with keratinolytic activity secreted by *Coccidioides immitis*. Mycopathologia. 2008;166:35–40.
128. López SN, Castelli MV, de Campos F, Corrêa R, Cechinel Filho V, Yunes RA, et al. *In vitro* antifungal properties structure-activity relationships and studies on the mode of action of N-phenyl, N-aryl, N-phenylalkyl maleimides and related compounds. Arzneimittelforschung. 2005;55:123–32.
129. López SN, Ramallo IA, Sierra MG, Zacchino SA, Furlan RL. Chemically engineered extracts as an alternative source of bioactive natural product-like compounds. Proc Natl Acad Sci USA. 2007;104:441–4.
130. Loures FV, Pina A, Felonato M, Calich VLG. TLR2 is a negative regulator of Th17 cells and tissue pathology in a pulmonary model of fungal infection. J Immunol. 2009;183:1279–90.
131. Loyola W, Gaziri DA, Gaziri LC, Felipe I. Concanavalin A enhances phagocytosis and killing of *Candida albicans* by mice peritoneal neutrophils and macrophages. FEMS Immunol Med Microbiol. 2002;33:201–8.
132. Machado AP, Regis Silva MR, Fischman O. Local phagocytic responses after murine infection with different forms of *Fonsecaea pedrosoi* and sclerotic bodies originating from an inoculum of conidiogenous cells. Mycoses. 2009. Nov 18. [Epub ahead of print].
133. Madrid H, Cano J, Gené J, Bonifaz A, Toriello C, Guarro J. *Sporothrix globosa*, a pathogenic fungus with widespread geographical distribution. Rev Iberoam Micol. 2009;26:218–22.
134. Maffei CM, Mirels LF, Sobel RA, Clemons KV, Stevens DA. Cytokine and inducible nitric oxide synthase mRNA expression during experimental murine cryptococcal meningoencephalitis. Infect Immun. 2004;72:2338–49.
135. Magnani T, Soriani FM, Martins VP, Nascimento AM, Tudella VG, Curti C, et al. Cloning and functional expression of the mitochondrial alternative oxidase of *Aspergillus fumigatus* and its induction by oxidative stress. FEMS Microbiol Lett. 2007;271:230–8.
136. Malavazi I, da Silva Ferreira ME, Soriani FM, Dinamarco TM, Savoldi M, Uyemura SA, et al. Phenotypic analysis of genes whose mRNA accumulation is dependent on calcineurin in *Aspergillus fumigatus*. Fungal Genet Biol. 2009;46:791–802.
137. Maluf ML, Takahachi G, Svidzinski TI, Xander P, Apitz-Castro R, Bersani-Amado CA, et al. Antifungal activity of ajoene on experimental murine paracoccidioidomycosis. Rev Iberoam Micol. 2008;25:163–6.
138. Mamoni RL, Blotta MH. Kinetics of cytokines and chemokines gene expression distinguishes *Paracoccidioides brasiliensis* infection from disease. Cytokine. 2005;32:20–9.
139. Mamoni RL, Nouér SA, Oliveira SJ, Musatti CC, Rossi CL, Camargo ZP, et al. Enhanced production of specific IgG4, IgE, IgA and TGF-beta in sera from patients with the juvenile form of paracoccidioidomycosis. Med Mycol. 2002;40:153–9.
140. Maricato JT, Batista WL, Kioshima ES, Feitosa LS, Brito RR, Goldman GH, et al. The *Paracoccidioides brasiliensis* GP70 antigen is encoded by a putative member of the flavoproteins monooxygenase family. Fungal Genet Biol. 2010;47:179–89.
141. Marques AF, da Silva MB, Juliano MA, Travassos LR, Taborda CP. Peptide immunization as an adjuvant to chemotherapy in mice challenged intratracheally with virulent yeast cells of *Paracoccidioides brasiliensis*. Antimicrob Agents Chemother. 2006;50:2814–9.
142. Marques AF, da Silva MB, Juliano MA, Muñoz JE, Travassos LR, Taborda CP. Additive effect of P10 immunization and chemotherapy in anergic mice challenged intratracheally with virulent yeasts of *Paracoccidioides brasiliensis*. Microbes Infect. 2008;10:1251–8.
143. Marques ER, Ferreira ME, Drummond RD, Felix JM, Menossi M, Savoldi M, et al. Identification of genes preferentially expressed in the pathogenic yeast phase of *Paracoccidioides brasiliensis*, using suppression subtraction hybridization and differential macroarray analysis. Mol Genet Genomics. 2004;271:667–77.
144. Marques Mello L, Silva-Vergara ML, Rodrigues Jr V. Patients with active infection with *Paracoccidioides brasiliensis* present a Th2 immune response characterized by high interleukin-4 and interleukin-5 production. Hum Immunol. 2002;63:149–54.
145. Martins LF, Montero-Lomeli M, Masuda CA, Fortes FS, Previato JO, Mendoca-Previato L. Lithium-mediated suppression of morphogenesis and growth in *Candida albicans*. FEMS Yeast Res. 2008;8:615–21.
146. Martins VP, Soriani FM, Magnani T, Tudella VG, Goldman GH, Curti C, et al. Mitochondrial function in the yeast form of the pathogenic fungus *Paracoccidioides brasiliensis*. J Bioenerg Biomembr. 2008;40:297–305.
147. Matsuo AL, Carmona AK, Silva LS, Cunha CE, Nakayasu ES, Almeida IC, et al. C-Npys (S-3-nitro-2-pyridinesulfenyl) and peptide derivatives can inhibit a serine-thiol proteinase activity from *Paracoccidioides brasiliensis*. Biochem Biophys Res Commun. 2007;355:1000–5.
148. Matsuo AL, Tersariol II, Kobata SI, Travassos LR, Carmona AK, Puccia R. Modulation of the exocellular serine-thiol proteinase activity of *Paracoccidioides brasiliensis* by neutral polysaccharides. Microbes Infect. 2006;8:84–91.
149. Matute DR, McEwen JG, Puccia R, Montes BA, San-Blas G, Bagagli E, et al. Cryptic speciation and recombination in the fungus *Paracoccidioides brasiliensis* as revealed by gene genealogies. Mol Biol Evol. 2006;23:65–73.
150. Matute DR, Quesada-Ocampo LM, Rauscher JT, McEwen JG. Evidence for positive selection in putative virulence factors within the *Paracoccidioides brasiliensis* species complex. PLoS Negl Trop Dis. 2008;2:e296.
151. Matute DR, Sepulveda VE, Quesada LM, Goldman GH, Taylor JW, Restrepo A, et al. Microsatellite analysis of three phylogenetic species of *Paracoccidioides brasiliensis*. J Clin Microbiol. 2006;44:2153–7.
152. Matute DR, Torres IP, Salgado-Salazar C, Restrepo A, McEwen JG. Background selection at the chitin synthase II (chs2) locus in *Paracoccidioides brasiliensis* species complex. Fungal Genet Biol. 2007;44:357–67.
153. Maza PK, Strauss AH, Toledo MS, Takahashi HK, Suzuki E. Interaction of epithelial cell membrane rafts with *Paracoccidioides brasiliensis* leads to fungal adhesion and Src-family kinase activation. Microbes Infect. 2008;10: 540–7.
154. Mazo Fávero Gimenes V, Da Glória de Souza M, Ferreira KS, Marques SG, Gonçalves AG, Vagner de Castro Lima Santos D, et al. Cytokines and lymphocyte proliferation in patients with different clinical forms of chromoblastomycosis. Microbes Infect. 2005;7:708–13.
155. Medeiros AI, Bonato VL, Malheiro A, Dias AR, Silva CL, Faccioli LH. *Histoplasma capsulatum* inhibits apoptosis and Mac-1 expression in leucocytes. Scand J Immunol. 2002;56:392–8.
156. Medeiros AI, Malheiro A, Jose PJ, Conroy DM, Williams TJ, Faccioli LH. Differential release of MIP-1alpha and eotaxin during infection of mice by *Histoplasma capsulatum* or inoculation of beta-glucan. Inflamm Res. 2004;53:351–4.
157. Medeiros AI, Sá-Nunes A, Turato WM, Secatto A, Frantz FG, Sorgi CA, et al. Leukotrienes are potent adjuvant during fungal infection: effects on memory T cells. J Immunol. 2008;181:8544–51.
158. Medzitov R. Recognition of microorganisms and activation of the immune response. Nat Rev Immunol. 2007;6:33–43.
159. Meléndez Gómez CM, Kouznetsov VV, Sortino MA, Alvarez SL, Zacchino SA. *In vitro* antifungal activity of polyfunctionalized 2-(hetero)arylquinolines prepared through imino Diels-Alder reactions. Bioorg Med Chem. 2008;16:7908–20.
160. Melo AS, Colombo AL, Arthington-Skaggs BA. Paradoxical growth effect of caspofungin observed on biofilms and planktonic cells of five different *Candida* species. Antimicrob Agents Chemother. 2007;51:3081–8.
161. Mendes A, Mores AU, Carvalho AP, Rosa RT, Samaranayake LP, Rosa EA. *Candida albicans* biofilms produce more secreted aspartyl protease than the planktonic cells. Biol Pharm Bull. 2007;30:1813–5.

162. Mesa-Arango AC, del Rocío Reyes-Montes M, Pérez-Mejía A, Navarro-Barranco H, Souza V, Zúñiga G, et al. Phenotyping and genotyping of *Sporothrix schenckii* isolates according to geographic origin and clinical form of sporotrichosis. J Clin Microbiol. 2002;40:3004–11.
163. Meyer W, Castañeda A, Jackson S, Huynh M, Castañeda E, IberoAmerican Cryptococcal Study Group. Molecular typing of IberoAmerican *Cryptococcus neoformans* isolates. Emerg Infect Dis. 2003;9:189–95.
164. Milani G, Jarmuszkiewicz W, Sluse-Goffart CM, Schreiber AZ, Vercesi AE, Sluse FE. Respiratory chain network in mitochondria of *Candida parapsilosis*: ADP/O appraisal of the multiple electron pathways. FEBS Lett. 2001;508:231–5.
165. Molinari-Madlum EEWI, Felipe MSS, Soares CMA. Virulence of *Paracoccidioides brasiliensis* isolates can be correlated to groups defined by random amplified polymorphic DNA analysis. Med Mycol. 1999;37:269–76.
166. Morais FV, Barros TF, Fukada MK, Cisalpino PS, Puccia R. Polymorphism in the gene coding for the immunodominant antigen gp43 from the pathogenic fungus *Paracoccidioides brasiliensis*. J Clin Microbiol. 2000;38:3960–6.
167. Mora-Montes HM, Bader O, López-Romero E, Zinker S, Ponce-Noyola P, Hube B, et al. Kex2 protease converts the endoplasmic reticulum alpha1,2-mannosidase of *Candida albicans* into a soluble cytosolic form. Microbiology. 2008;154:3782–94.
168. Mora-Montes HM, Bates S, Netea MG, Díaz-Jiménez DF, López-Romero E, Zinker S, et al. Endoplasmic reticulum alpha-glycosidases of *Candida albicans* are required for N-glycosylation, cell wall integrity, and normal host-fungus interaction. Eukaryot Cell. 2007;6:2184–93.
169. Mora-Montes HM, López-Romero E, Zinker S, Ponce-Noyola P, Flores-Carreón A. Heterologous expression and biochemical characterization of an alpha1,2-mannosidase encoded by the *Candida albicans* MNS1 gene. Mem Inst Oswaldo Cruz. 2008;103:724–30.
170. Moreira AP, Dias-Melicio LA, Peraçoli MT, Calvi SA, Victoriano de Campos Soares AM. Killing of *Paracoccidioides brasiliensis* yeast cells by IFN-gamma and TNF-alpha activated murine peritoneal macrophages: evidence of H_2O_2 and NO effector mechanisms. Mycopathologia. 2008;166:17–23.
171. Moreira-Oliveira MS, Mikami Y, Miyaji M, Imai T, Schreiber AZ, Moretti ML. Diagnosis of candidemia by polymerase chain reaction and blood culture: prospective study in a high-risk population and identification of variables associated with development of candidemia. Eur J Clin Microbiol Infect Dis. 2005;24:721–6.
172. Mores AU, Souza RD, Cavalca L, de Paula E, Carvalho A, Gursky LC, et al. Enhancement of secretory aspartyl protease production in biofilms of *Candida albicans* exposed to sub-inhibitory concentrations of fluconazole. Mycoses. 2009 Oct 28. [Epub ahead of print].
173. Murgich J, Abanero J, San-Blas G. Molecular modeling of poly-alfa and beta-1-3-glucans, major components of *Paracoccidiodes brasiliensis* cell wall. In: Taft CA, editor. *Current Trends in Biotechnology*. Kerala, India: Research Signpost; 2005. p. 67–82.
174. Murgich J, Franco H, San-Blas G. Molecular modelling of echinocandin-type antibiotics using molecular mechanics and density functional theory calculations. In: San-Blas G, Calderone R, editors. *Pathogenic Fungi: Host Interactions and Emerging Strategies for Control*. Wymondham, Norfolk, UK: Caister Academic Press; 2004. p. 449–66.
175. Murgich J, Franco HJ, San-Blas G. Topology of charge density of flucytosine and related molecules and characteristics of their bond charge distributions. J Phys Chem A. 2006;110:10106–15.
176. Nascimento AM, Goldman GH, Park S, Marras SA, Delmas G, Oza U, et al. Multiple resistance mechanisms among *Aspergillus fumigatus* mutants with high-level resistance to itraconazole. Antimicrob Agents Chemother. 2003;47:1719–26.
177. Nascimento FRF, Rodriguez D, Russo M. Dual role for nitric oxide in paracoccidioidomycosis: essential for resistence, but overproduction associated with susceptibility. J Immunol. 2002;168:4593–600.
178. Neto BR, Silva JD, Mendes-Giannini MJ, Lenzi HL, de Almeida Soares CM, Pereira M. The malate synthase of *Paracoccidioides brasiliensis* is a linked surface protein that behaves as an anchorless adhesin. BMC Microbiol. 2009;9:272.
179. Nimrichter L, Barreto-Bergter E, Mendonça-Filho RR, Kneipp LF, Mazzi MT, Salve P, et al. A monoclonal antibody to glucosylceramide inhibits the growth of *Fonsecaea pedrosoi* and enhances the antifungal action of mouse macrophages. Microbes Infect. 2004;6:657–65.
180. Nimrichter L, Cerqueira MD, Leitão EA, Miranda K, Nakayasu ES, Almeida SR, et al. Structure, cellular distribution, antigenicity, and biological functions of *Fonsecaea pedrosoi* ceramide monohexosides. Infect Immun. 2005;73:7860–8.
181. Niño-Vega GA, Calcagno AM, San-Blas G, San-Blas F, Gooday GW, Gow NA. RFLP analysis reveals marked geographical isolation between strains of *Paracoccidioides brasiliensis*. Med Mycol. 2000;38:437–41.
182. Niño-Vega GA, Carrero L, San-Blas G. Isolation of the CHS4 gene of *Paracoccidioides brasiliensis* and its accommodation in a new class of chitin synthases. Med Mycol. 2004;42:51–7.
183. Niño-Vega GA, Munro CA, San-Blas G, Gooday GW, Gow NA. Differential expression of chitin synthase genes during temperature-induced dimorphic transitions in *Paracoccidioides brasiliensis*. Med Mycol. 2000;38:31–9.
184. Niño-Vega GA, Sorais F, Calcagno AM, Ruiz-Herrera J, Martínez-Espinoza AD, San-Blas G. Cloning and expression analysis of the ornithine decarboxylase gene (PbrODC) of the pathogenic fungus *Paracoccidioides brasiliensis*. Yeast. 2004;21:211–8.
185. Niño-Vega GA, Sorais F, San-Blas G. Transcription levels of CHS5 and CHS4 genes in *Paracoccidioides brasiliensis* mycelial phase, respond to alterations in external osmolarity, oxidative stress and glucose concentration. Mycol Res. 2009;113:1091–6.
186. Nishikaku AS, Molina RF, Ribeiro LC, Scavone R, Albe BP, Cunha CS, et al. Nitric oxide participation in granulomatous response induced by *Paracoccidioides brasiliensis* infection in mice. Med Microbiol Immunol. 2009;198:123–35.
187. Nishikaku AS, Ribeiro LC, Molina RFS, Albe BP, Cunha Cda S, Burger E. Matrix metalloproteinases with gelatinolytic activity induced by *Paracoccidioides brasiliensis* infection. Int J Exp Pathol. 2009;90:527–37.
188. Nishikaku AS, Scavone R, Molina RFS, Albe BP, Cunha C, da S, Burger E. Osteopontin involvement in granuloma formation and in the severity of *Paracoccidioides brasiliensis* infection. Med Mycology. 2009;47:495–507.
189. Nosanchuk JD, Nimrichter L, Casadevall A, Rodrigues ML. A role for vesicular transport of macromolecules across cell walls in fungal pathogenesis. Commun Integr Biol. 2008;1:37–9.
190. Nunes LR, Costa de Oliveira R, Leite DB, da Silva VS, dos Reis Marques E, da Silva Ferreira ME, et al. Transcriptome analysis of *Paracoccidioides brasiliensis* cells undergoing mycelium-to-yeast transition. Eukaryot Cell. 2005;4:2115–28.
191. Olivares LR, Martínez KM, Cruz RM, Rivera MA, Meyer W, Espinosa RA, et al. Genotyping of Mexican *Cryptococcus neoformans* and *C. gattii* isolates by PCR-fingerprinting. Med Mycol. 2009;47:713–21.
192. Oliveira DL, Nimrichter L, Miranda K, Frases S, Faull KF, Casadevall A, et al. *Cryptococcus neoformans* cryoultramicrotomy and vesicle fractionation reveals an intimate association between membrane lipids and glucuronoxylomannan. Fungal Genet Biol. 2009;46:956–63.
193. Palmeira VF, Kneipp LF, Alviano CS, dos Santos AL. Secretory aspartyl peptidase activity from mycelia of the human fungal pathogen *Fonsecaea pedrosoi*: effect of HIV aspartyl proteolytic inhibitors. Res Microbiol. 2006;157:819–26.
194. Palmeira VF, Kneipp LF, Alviano CS, dos Santos AL. The major chromoblastomycosis fungal pathogen, *Fonsecaea pedrosoi*, extracellularly releases proteolytic enzymes whose expression is modulated by culture medium composition: implications on the fungal development and cleavage of key's host structures. FEMS Immunol Med Microbiol. 2006;46:21–9.
195. Panagio LA, Tristao FS, Moreira AP, Pereira MS, Cavassani KA, Milanezi CM, et al. Role of interleukin (IL)-18 in experimental paracoccidioidomycosis. Med Mycol. 2008;46:435–42.
196. Pantoja-Hernández MA, Muñoz-Sánchez CI, Guevara-González RG, Botello-Alvarez E, González-Chavira MM, Torres-Pacheco I, et al. Expression of ornithine decarboxylase of *Coccidioides immitis* in three *Escherichia coli* strains carrying the lambda DE3 lysogen and an *E. coli* EWH319 strain odc-null mutant. Biotechnol Lett. 2004;26:75–8.
197. Parra C, González JM, Castañeda E, Fiorentino S. Anti-glucuronoxylomannan IgG1 specific antibodies production in *Cryptococcus neoformans* resistant mice. Biomedica. 2005;25:110–9.
198. Parra-Ortega B, Cruz-Torres H, Villa-Tanaca L, Hernández-Rodríguez C. Phylogeny and evolution of the aspartyl protease family from clinically relevant *Candida* species. Mem Inst Oswaldo Cruz. 2009;104:505–12.
199. Peraçoli MTS, Kurokawa CS, Calvi S, Mendes RP, Pereira PC, Marques SA, et al. Production of pro-and anti-inflammatory cytokine by monocytes from patients with paracoccidioidomycosis. Microbes Infect. 2003;5:413–8.
200. Pereira CB, Bueno FL, Dias AL, Brigagão MR, Paula CR, Siqueira AM. Evaluation of laccases and melanization in clinical and environmental *Cryptococcus neoformans* samples by non-denaturing PAGE. J Med Microbiol. 2009;58:563–6.
201. Pereira M, Felipe MS, Brígido MM, Soares CM, Azevedo MO. Molecular cloning and characterization of a glucan synthase gene from the human pathogenic fungus *Paracoccidioides brasiliensis*. Yeast. 2000;16:451–62.
202. Pérez-Blanco M, Valles RH, Zeppenfeldt GF, Apitz-Castro R. Ajoene and 5-fluorouracil in the topical treatment of *Cladophialophora carrionii* chromoblastomycosis in humans: a comparative open study. Med Mycol. 2003;41:517–20.
203. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20:133–63.
204. Pfaller MA, Diekema DJ, Messer SA, Boyken L, Hollis RJ. Activities of fluconazole and voriconazole against 1,586 recent clinical isolates of *Candida* species determined by Broth microdilution, disk diffusion, and Etest methods: report from the ARTEMIS Global Antifungal Susceptibility Program, 2001. J Clin Microbiol. 2003;41:1440–6.
205. Pinto MR, Rodrigues ML, Travassos LR, Haido RM, Wait R, Barreto-Bergter E. Characterization of glucosylceramides in *Pseudallescheria boydii* and their involvement in fungal differentiation. Glycobiology. 2002;12:251–60.
206. Pinto PM, Resende MA, Koga-Ito CY, Ferreira JA, Tendler M. rDNA-RFLP identification of *Candida* species in immunocompromised and seriously diseased patients. Can J Microbiol. 2004;50:514–20.
207. Polonelli L, Casadevall A, Han Y, Bernardis F, Kirkland TN, Matthews RC, et al. The efficacy of acquired humoral and cellular immunity in the prevention and therapy of experimental fungal infections. Med Mycol. 2000;38 Suppl 1:281–92.
208. Prado M, da Silva MB, Laurenti R, Travassos LR, Taborda CP. Mortality due to systemic mycoses as a primary cause of death or in association with AIDS in Brazil: a review from 1996 to 2006. Mem Inst Oswaldo Cruz. 2009;104:513–21.
209. Reis BS, Fernandes VC, Martins EM, Serakides R, Goes AM. Protective immunity induced by rPb27 of *Paracoccidioides brasiliensis*. Vaccine. 2008;26:5461–9.

210. Reis RS, Almeida-Paes R, Muniz Mde M, Tavares PM, Monteiro PC, Schubach TM, et al. Molecular characterisation of *Sporothrix schenckii* isolates from humans and cats involved in the sporotrichosis epidemic in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz. 2009;104:769–74.
211. Renna MS, Correa SG, Porporatto C, Figueredo CM, Aoki MP, Paraje MG, et al. Hepatocellular apoptosis during *Candida albicans* colonization: involvement of TNF-alpha and infiltrating Fas-L positive lymphocytes. Int Immunol. 2006;18:1719–28.
212. Reyna-López GE, Ruiz-Herrera J. Specificity of DNA methylation changes during fungal dimorphism and its relationship to polyamines. Curr Microbiol. 2004;48:118–23.
213. Rittershaus PC, Kechichian TB, Allegood JC, Merrill Jr AH, Hennig M, Luberto C, et al. Glucosylceramide synthase is an essential regulator of pathogenicity of *Cryptococcus neoformans*. J Clin Investig. 2006;116:1651–9.
214. Rodero L, Davel G, Soria M, Vivot W, Córdoba S, Canteros CE, et al. Estudio multicéntrico de fungemia debida a levaduras en Argentina. Rev Argent Microbiol. 2005;37:189–95.
215. Rodero L, Mellado E, Rodriguez AC, Salve A, Guelfand L, Cahn P, et al. G484S amino acid substitution in lanosterol 14-alpha demethylase (ERG11) is related to fluconazole resistance in a recurrent *Cryptococcus neoformans* clinical isolate. Antimicrob Agents Chemother. 2003;47:3653–6.
216. Rodrigues ML, Nakayasu ES, Oliveira DL, Nimrichter L, Nosanchuk JD, Almeida IC, et al. Extracellular vesicles produced by *Cryptococcus neoformans* contain protein components associated with virulence. Eukaryot Cell. 2008;7:58–67.
217. Rodrigues ML, Nimrichter L, Oliveira DL, Frases S, Miranda K, Zaragoza O, et al. Vesicular polysaccharide export in *Cryptococcus neoformans* is a eukaryotic solution to the problem of fungal trans-cell wall transport. Eukaryot Cell. 2007;6:48–59.
218. Rodrigues ML, Shi L, Barreto-Bergter E, Nimrichter L, Farias SE, Rodrigues EG, et al. Monoclonal antibody to fungal glucosylceramide protects mice against lethal *Cryptococcus neoformans* infection. Clin Vaccine Immunol. 2007;14:1372–6.
219. Rodríguez-Brito S, Niño-Vega G, San-Blas G. Caspofungin affects growth of *Paracoccidioides brasiliensis* in both morphological phases. Antimicrob Agents Chemother. 2010;54:5391–4.
220. Romano CC, Mendes-Giannini MJ, Duarte AJ, Benard G. The role of interleukin-10 in the differential expression of interleukin-12p70 and its beta2 receptor on patients with active or treated paracoccidioidomycosis and healthy infected subjects. Clin Immunol. 2005;114:86–94.
221. Rosas-Hernández LL, Juárez-Reyes A, Arroyo-Helguera OE, de las Peñas A, Pan SJ, Cormack BP, et al. yKu70/yKu80 and Rif1 regulate silencing differentially at telomeres in *Candida glabrata*. Eukaryot Cell. 2008;7:2168–78.
222. Ruiz-Baca E, Toriello C, Perez-Torres A, Sabanero-Lopez M, Villagomez-Castro JC, Lopez-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.
223. Ruiz-Herrera J, Elorza MV, Valentín E, Sentandreu R. Molecular organization of the cell wall of *Candida albicans* and its relation to pathogenicity. FEMS Yeast Res. 2006;6:14–29.
224. Ruiz-Herrera J, González-Prieto JM, Ruiz-Medrano R. Evolution and phylogenetic relationships of chitin synthases from yeasts and fungi. FEMS Yeast Res. 2002;1:247–56.
225. Ruiz-Herrera J, San-Blas G. Chitin synthesis as target for antifungal drugs. Current Drug Target Infect Dis. 2003;3:77–91.
226. Ruy F, Vercesi AE, Kowaltowski AJ. Inhibition of specific electron transport pathways leads to oxidative stress and decreased *Candida albicans* proliferation. J Bioenerg Biomembr. 2006;38:129–35.
227. Sá-Nunes A, Medeiros AI, Nicolete R, Frantz FG, Panunto-Castelo A, Silva CL, et al. Efficacy of cell-free antigens in evaluating cell immunity and inducing protection in a murine model of histoplasmosis. Microbes Infect. 2005;7:584–92.
228. San-Blas G, Niño-Vega G. Morphogenesis of agents of endemic mycoses. In: San-Blas G, Calderone R, editors. *Pathogenic Fungi: Structural Biology and Taxonomy*. Wymondham, Norfolk, UK: Caister Academic Press; 2004. p. 167–220.
229. San-Blas G, Niño-Vega G. New approaches in the diagnosis of medically relevant mycoses: Fungal identification by molecular techniques. In: San-Blas G, Calderone R, editors. *Pathogenic Fungi: Insights In Molecular Biology*. Wymondham, Norfolk, UK: Caister Academic Press;; 2008. p. 225–57.
230. San-Blas G, Niño-Vega G, Barreto L, Hebeler-Barbosa F, Bagagli E, Olivero de Briceño R, et al. Primers for the clinical detection of *Paracoccidioides brasiliensis*. J Clin Microbiol. 2005;49:4255–7.
231. San-Blas G, Niño-Vega G, Iturriaga T. *Paracoccidioides brasiliensis* and PCM: Molecular approaches to morphogenesis, diagnosis, epidemiology, taxonomy and genetics. Med Mycol. 2002;40:225–42.
232. San-Blas G, Sorais F, San-Blas F, Ruiz-Herrera J. Ornithine decarboxylase in *Paracoccidioides brasiliensis*. Arch Microbiol. 1996;165:311–6.
233. San-Blas G, Urbina JA, Marchán E, Contreras LM, Sorais F, San-Blas F. Inhibition of *Paracoccidioides brasiliensis* by ajoene is associated with blockade of phosphatidylcholine biosynthesis. Microbiology. 1997;143:1583–6.
234. Sassá MF, Saturi AE, Souza LF, Ribeiro LC, Sgarbi DB, Carlos IZ. Response of macrophage Toll-like receptor 4 to a *Sporothrix schenckii* lipid extract during experimental sporotrichosis. Immunology. 2009;128:301–9.
235. Segato F, Nozawa SR, Rossi A, Martinez-Rossi NM. Over-expression of genes coding for proline oxidase, riboflavin kinase, cytochrome c oxidase and an MFS transporter induced by acriflavin in *Trichophyton rubrum*. Med Mycol. 2008;46:135–9.
236. Silva V, Alvarado D, Díaz MC. Antifungal susceptibility of 50 *Candida* isolates from invasive mycoses in Chile. Med Mycol. 2004;42:283–5.
237. Soares RM, Costa e Silva-Filho F, Rozental S, Angluster J, de Souza W, Alviano CS, et al. Anionogenic groups and surface sialoglycoconjugate structures of yeast forms of the human pathogen *Paracoccidioides brasiliensis*. Microbiology. 1998;144:309–14.
238. Soares RM, de A, Soares RM, Alviano DS, Angluster J, Alviano CS, Travassos LR. Identification of sialic acids on the cell surface of *Candida albicans*. Biochim Biophys Acta. 2000;1474:262–8.
239. Sorais F, Barreto L, Leal JA, Bernabé M, San-Blas G, Niño-Vega GA. Cell wall glucan synthases and GTPases in *Paracoccidioides brasiliensis*. Med Mycol. 2010;48:35–47.
240. Sorais F, Niño-Vega G, San-Blas G. Mecanismos de degradación de la ornitina descarboxilasa fúngica. Rev Iberoam Micol. 2003;20:1–5.
241. Sorgi CA, Secatto A, Fontanari C, Turato WM, Belangér C, de Medeiros AI, et al. *Histoplasma capsulatum* cell wall {beta}-glucan induces lipid body formation through CD18, TLR2, and dectin-1 receptors: correlation with leukotriene B4 generation and role in HIV-1 infection. J Immunol. 2009;182:4025–35.
242. Soriani FM, Malavazi I, da Silva Ferreira ME, Savoldi M, Von Zeska Kress MR, de Souza Goldman MH, et al. Functional characterization of the *Aspergillus fumigatus* CRZ1 homologue. CrzA Mol Microbiol. 2008;67:1274–91.
243. Sortino M, Cechinel Filho V, Corrêa R, Zacchino S. N-Phenyl and N-phenylalkyl-maleimides acting against *Candida* spp.: time-to-kill, stability, interaction with maleamic acids. Bioorg Med Chem. 2008;16:560–8.
244. Sousa MG, de Maria Pedrozo e Silva Azevedo C, Nascimento RC, Ghosn EE, Santiago KL, Noal V, et al. *Fonsecaea pedrosoi* infection induces differential modulation of co stimulatory molecules and cytokines in monocytes from patients with severe and mild forms of chromoblastomycosis. J Leukoc Biol. 2008;84:864–70.
245. Sousa MG, Ghosn EE, Nascimento RC, Bomfim GF, Noal V, Santiago K, et al. Monocyte-derived dendritic cells from patients with severe forms of chromoblastomycosis induce CD4+ T cell activation in vitro. Clin Exp Immunol. 2009;156:117–25.
246. Souza TF, Scroferneker ML, Costa JM, Carissimi M, Corbellini VA. Secretion of five extracellular enzymes by strains of chromoblastomycosis agents. Rev Inst Med Trop Sao Paulo. 2008;50:269–72.
247. Souto G, Giacometti R, Silberstein S, Giasson L, Cantore ML, Passeron S. Expression of TPK1 and TPK2 genes encoding PKA catalytic subunits during growth and morphogenesis in *Candida albicans*. Yeast. 2006;23:591–603.
248. Souto JT, Aliberti JC, Campanelli AP, Livonesi MC, Maffei CM, Ferreira BR, et al. Chemokine production and leukocyte recruitment to the lungs of *Paracoccidioides brasiliensis*-infected mice is modulated by interferon-gamma. Am J Pathol. 2003;163:583–90.
249. Stevens DA. Frequency of paradoxical effect with caspofungin in *Candida albicans*. Eur J Clin Microbiol Infect Dis. 2009;28:717.
250. Suzuki E, Tanaka AK, Toledo MS, Levery SB, Straus AH, Takahashi HK. Trypanosomatid and fungal glycolipids and sphingolipids as infectivity factors and potential targets for development of new therapeutic strategies. Biochim Biophys Acta. 2008;1780:362–9.
251. Taborda CP, Juliano MA, Puccia R, Franco M, Travassos LR. Mapping of the T-cell epitope in the major 43-kilodalton glycoprotein of *Paracoccidioides brasiliensis* which induces a Th-1 response protective against fungal infection in BALB/c mice. Infect Immun. 1998;66:786–93.
252. Taborda CP, Nakaie CR, Cilli EM, Rodrigues EG, Silva LS, Franco MF, et al. Synthesis and immunological activity of a branched peptide carrying the T-cell epitope of gp43, the major exocellular antigen of *Paracoccidioides brasiliensis*. Scand J Immunol. 2004;59:58–65.
253. Takayama A, Itano EN, Sano A, Ono MA, Kamei K. An atypical *Paracoccidioides brasiliensis* clinical isolate based on multiple gene analysis. Med Mycol. 2010;48:64–72.
254. Tavares AH, Silva SS, Dantas A, Campos EG, Andrade RV, Maranhão AQ, et al. Early transcriptional response of *Paracoccidioides brasiliensis* upon internalization by murine macrophages. Microbes Infect. 2007;9:583–90.
255. Tavian EG, Dias-Melicio LA, Acorci MJ, Graciani AP, Peraçoli MT, Soares AM. Interleukin-15 increases *Paracoccidioides brasiliensis* killing by human neutrophils. Cytokine. 2008;41:48–53.
256. Taylor ML, Duarte-Escalante E, Pérez A, Zenteno E, Toriello C. *Histoplasma capsulatum* yeast cells attach and agglutinate human erythrocytes. Med Mycol. 2004;42:287–92.
257. Teixeira MM, Theodoro RC, de Carvalho MJ, Fernandes L, Paes HC, Hahn RC, et al. Phylogenetic analysis reveals a high level of speciation in the *Paracoccidioides* genus. Mol Phylogenet Evol. 2009;52:273–83.
258. Teixeira PA, de Castro RA, Nascimento RC, Tronchin G, Torres AP, Lazéra M, et al. Cell surface expression of adhesins for fibronectin correlates with virulence in *Sporothrix schenckii*. Microbiology. 2009;155:3730–8.
259. Teixeira de Sousa M, da G, Ghosn EE, Almeida SR. Absence of CD4+ T cells impairs host defence of mice infected with *Fonsecaea pedrosoi*. Scand J Immunol. 2006;64:595–600.
260. Thomaz L, Apitz-Castro R, Marques AF, Travassos LR, Taborda CP. Experimental PCM: alternative therapy with ajoene, compound from *Allium sativum*, associated with sulfamethoxazole/trimethoprim. Med Mycol. 2008;46:113–8.
261. Toledo MS, Levery SB, Bennion B, Guimaraes LL, Castle SA, Lindsey R, et al. Analysis of glycosylinositol phosphorylceramides expressed by the opportunistic mycopathogen *Aspergillus fumigatus*. J Lipid Res. 2007;48:1801–24.

262. Toledo MS, Levery SB, Glushka J, Straus AH, Takahashi HK. Structure elucidation of sphingolipids from the mycopathogen *Sporothrix schenckii*: identification of novel glycosylinositol phosphorylceramides with core manalpha1–6Ins linkage. Biochem Biophys Res Commun. 2001;280: 19–24.
263. Toledo MS, Levery SB, Straus AH, Takahashi HK. Dimorphic expression of cerebrosides in the mycopathogen *Sporothrix schenckii*. J Lipid Res. 2000;41:797–806.
264. Toledo MS, Levery SB, Straus AH, Takahashi HK. Sphingolipids of the mycopathogen *Sporothrix schenckii*: identification of a glycosylinositol phosphorylceramide with novel core GlcNH(2)alpha1–2Ins motif. FEBS Lett. 2001;493:50–6.
265. Tomazett PK, Cruz AH, Bonfim SM, Soares CM, Pereira M. The cell wall of *Paracoccidioides brasiliensis*: insights from its transcriptome. Genet Mol Res. 2005;4:309–25.
266. Torres FA, Vilaça R, Pepe de Moraes LM, Reis VC, Felipe MS. Expression of a kexin-like gene from the human pathogenic fungus *Paracoccidioides brasiliensis* in *Saccharomyces cerevisiae*. Med Mycol. 2008;46:385–8.
267. Torres I, García AM, Hernández O, González A, McEwen JG, Restrepo A, et al. Presence and expression of the mating type locus in *Paracoccidioides brasiliensis* isolates. Fungal Genet Biol. 2010;47:373–80.
268. Tosello ME, Biasoli MS, Luque AG, Magaró HM, Krapp AR. Oxidative stress response involving induction of protective enzymes in *Candida dubliniensis*. Med Mycol. 2007;45:535–40.
269. Travassos LR, Silva LS, Rodrigues EG, Conti S, Salati A, Magliani W, et al. Therapeutic activity of a killer peptide against experimental paracoccidioidomycosis. J Antimicrob Chemother. 2004;54:956–8.
270. Trilles L, Lazéra M, dos S, Wanke B, Oliveira RV, Barbosa GG, Nishikawa MM, et al. Regional pattern of the molecular types of *Cryptococcus neoformans* and *Cryptococcus gattii* in Brazil. Mem Inst Oswaldo Cruz. 2008;103: 455–62.
271. Tudella VG, Curti C, Soriani FM, Santos AC, Uyemura SA. *In situ* evidence of an alternative oxidase and an uncoupling protein in the respiratory chain of *Aspergillus fumigatus*. Int J Biochem Cell Biol. 2004;36:162–72.
272. Urbina JA, Visbal G, Contreras LM, McLaughlin G, Docampo R. Inhibitors of delta24(25) sterol methyltransferase block sterol synthesis and cell proliferation in *Pneumocystis carinii*. Antimicrob Agents Chemother. 1997;41:1428–32.
273. Urbina JA, Vivas J, Lazardi K, Molina J, Payares G, Piras MM, et al. Antiproliferative effects of delta 24(25) sterol methyl transferase inhibitors on *Trypanosoma* (*Schizotrypanum*) *cruzi*: *in vitro* and *in vivo* studies. Chemotherapy. 1996;42:294–307.
274. Urbina JM, Cortés JC, Palma A, López SN, Zacchino SA, Enriz RD, et al. Inhibitors of the fungal cell wall. Synthesis of 4-aryl-4-N-arylamine-1-butenes and related compounds with inhibitory activities on beta(1-3) glucan and chitin synthases. Bioorg Med Chem. 2000;8:691–8.
275. Vargas MLY, Castelli MV, Kouznetsov VV, Urbina GJM, López SN, Sortino M, et al. *In vitro* antifungal activity of new series of homoallylamines and related compounds with inhibitory properties of the synthesis of fungal cell wall polymers. Bioorg Med Chem. 2003;11:1531–50.
276. Venancio EJ, Daher BS, Andrade RV, Soares CM, Pereira IS, Felipe MS. The kex2 gene from the dimorphic and human pathogenic fungus *Paracoccidioides brasiliensis*. Yeast. 2002;19:1221–31.
277. Viani FC, Dos Santos JI, Paula CR, Larson CE, Gambale W. Production of extracellular enzymes by *Microsporum canis* and their role in its virulence. Med Mycol. 2001;39:463–8.
278. Vilela R, Mendoza L, Rosa PS, Belone AF, Madeira S, Opromolla DV, et al. Molecular model for studying the uncultivated fungal pathogen *Lacazia loboi*. J Clin Microbiol. 2005;43:3657–61.
279. Vilela R, Rosa PS, Belone AF, Taylor JW, Diório SM, Mendoza L. Molecular phylogeny of animal pathogen *Lacazia loboi* inferred from rDNA and DNA coding sequences. Mycol Res. 2009;113:851–7.
280. Villena SN, Pinheiro RO, Pinheiro CS, Nunes MP, Takiya CM, DosReis GA, et al. Capsular polysaccharides galactoxylomannan and glucuronoxylomannan from *Cryptococcus neoformans* induce macrophage apoptosis mediated by Fas ligand. Cell Microbiol. 2008;10:1274–85.
281. Visbal G, Alvarez A, Moreno B, San-Blas G. S-Adenosyl-L-methionine inhibitors delta(24)-sterol methyltransferase and delta(24(28))-sterol methylreductase as possible agents against *Paracoccidioides brasiliensis*. Antimicrob Agents Chemother. 2003;47:2966–70.
282. Visbal G, San-Blas G, Murgich J, Franco H. *Paracoccidioides brasiliensis*, PCM, and antifungal antibiotics. Curr Drug Targets Infect Disord. 2005;5:211–26.
283. Xiao L, Madison V, Chau AS, Loebenberg D, Palermo RE, McNicholas PM. Three-dimensional models of wild-type and mutated forms of cytochrome P450 14alpha-sterol demethylases from *Aspergillus fumigatus* and *Candida albicans* provide insights into posaconazole binding. Antimicrob Agents Chemother. 2004;48:568–74.
284. Yoshida S, Kasuga S, Hayashi N, Ushiroguchi T, Matsuura H, Nakagawa S. Antifungal activity of ajoene derived from garlic. Appl Environ Microbiol. 1987;53:615–7.
285. Zaga-Clavellina V, López GG, Estrada-Gutiérrez G, Martínez-Flores A, Maida-Claros R, Beltran-Montoya J, et al. Incubation of human chorioamniotic membranes with Candida albicans induces differential synthesis and secretion of interleukin-1beta, interleukin-6, prostaglandin E, and 92 kDa type IV collagenase. Mycoses. 2006;49:6–13.
286. Zambrano EA, Rodríguez I, Mendoza M, Santaella C, López M, Albornoz M. Regulation of serine-type exoproteinases by endogenous inhibitors present in exoantigens of the mycelial form of *Paracoccidioides brasiliensis*. Med Mycol. 2001;39:359–68.
287. Zambuzzi-Carvalho PF, Cruz AH, Santos-Silva LK, Goes AM, Soares CM, Pereira M. The malate synthase of *Paracoccidioides brasiliensis* Pb01 is required in the glyoxylate cycle and in the allantoin degradation pathway. Med Mycol. 2009;47:734–44.
288. Zanatta N, Alves SH, Coelho HS, Borchhardt DM, Machado P, Flores KM, et al. Synthesis, antimicrobial activity, and QSAR studies of furan-3-carboxamides. Bioorg Med Chem. 2007;15:1947–58.
289. Zelada A, Castilla R, Passeron S, Giasson L, Cantore ML. Interactions between regulatory and catalytic subunits of the *Candida albicans* cAMP-dependent protein kinase are modulated by autophosphorylation of the regulatory subunit. Biochim Biophys Acta. 2002;1542:73–81.
290. Zelada A, de Souza FS, Walz K, Giasson L, Passeron S. cDNA cloning, biochemical and phylogenetic characterization of beta- and beta'-subunits of *Candida albicans* protein kinase CK2. Yeast. 2003;20:471–8.
291. Zhou W, Song Z, Kanagasabai R, Liu J, Jayasimha P, Sinha A, et al. Mechanism-based enzyme inactivators of phytosterol biosynthesis. Molecules. 2004;9:185–203.