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VIRULENCE FACTORS IN FUNGI OF SYSTEMIC MYCOSES

Cilmery Suemi KUROKAWA (1), Maria Fátima SUGIZAKI (1) & Maria Terezinha Serrão PERAÇOLI (1)

SUMMARY

Pathogenic fungi that cause systemic mycoses retain several factors which allow their growth in adverse conditions provided by the host, leading to the establishment of the parasitic relationship and contributing to disease development. These factors are known as virulence factors which favor the infection process and the pathogenesis of the mycoses. The present study evaluates the virulence factors of pathogenic fungi such as *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum* and *Paracoccidioides brasiliensis* in terms of thermotolerance, dimorphism, capsule or cell wall components as well as enzyme production. Virulence factors favor fungal adhesion, colonization, dissemination and the ability to survive in hostile environments and elude the immune response mechanisms of the host. Both the virulence factors presented by different fungi and the defense mechanisms provided by the host require action and interaction of complex processes whose knowledge allows a better understanding of the pathogenesis of systemic mycoses.

KEYWORDS: Virulence factors; Systemic Mycosis; *Paracoccidioides brasiliensis; Histoplasma capsulatum; Coccidioides immitis; Blastomyces dermatitidis; Cryptococcus neoformans.*

INTRODUCTION

Mycologists estimate that there are 100,000 species of fungi in nature. These fungi inhabit different niches, a number of them are symbiotic and may live in commensalism, mutualism or parasitism with other organisms. However, only some of the fungal species are pathogenic to man, a fact that has led to several studies providing a better understanding of the relationship among parasite, host and virulence factors [14,93].

The symbiotic-parasitic relationship produces an infectious process leading to lesions of the host tissues and establishment of disease due to a direct imbalance in parasite-host interaction. The host provides conditions for growth that usually differ markedly from the ecological niche that the fungus normally inhabits. In order to survive in this new environment, potential pathogens must withstand high temperatures, hormonal influences and attacks by phagocytes cells of the immune system [93] (Figure 1).

This process of adaptation to a more resistant form to the new microenvironment frequently results in aggression to host tissues. Some fungi, such as dimorphic fungi, have a greater ability to grow in adverse conditions provided by the host, and to produce disease. This process called pathogenicity is considered to be the result of direct interaction between the pathogen and host. Several fungal factors may help in this relationship and are frequently studied being known as virulence factors [14,38].

For an organism to cause disease it must (1) enter the host, (2) multiply in host tissues, (3) resist or not stimulate host defense mechanisms, and (4) damage the host. The success of all these processes will depend on which virulence factor the fungus uses [14].

Some virulence factors are of obvious importance. For example, the ability of a fungus to grow at 37°C is a virulence factor for invasive fungi, representing the transition to a parastic form essential for the pathogenicity of dimorphic fungi [38]. It is worth pointing out that not all fungal products may be considered as virulence factors. An example is the production of chitinase and ß-glucanase by spherules of *Coccidioides immitis* during the transition from the mycelial to parasitic form. Chitinase and ß-glucanase can only be considered as virulence factors if a probable interaction of the above-mentioned proteins with the host is suggested [38].

Thermotolerance

The ability to survive and replicate at 37°C seems to be a common property of pathogenic fungi. This phenomenon, known as thermotolerance, is observed in *Cryptococcus neoformans*, *Histoplasma capsulatum* and *Sporothrix schenckii* [56,93]. Most isolates of *C. neoformans* var. *gattii* that do not grow efficiently at 37°C are not able to produce fatal infection in mice, whereas isolates of var. *neoformans* germinate and grow at 37°C producing lethal infection[93]. Low-virulence strains of *H. capsulatum* require more time for mycelium-to-yeast-phase transition at 37°C, whereas the more

(1) Departamento de Microbiologia e Imunologia, Instituto de Biociências, UNESP, Botucatu, SP, Brasil.
Correspondence to: Dra. Maria Terezinha Serrão Peraçoli. Departamento de Microbiologia e Imunologia, Instituto de Biociências de Botucatu, UNESP. 18618-000 Botucatu, SP, Brasil. e-mail: microimuno@laser.com.br

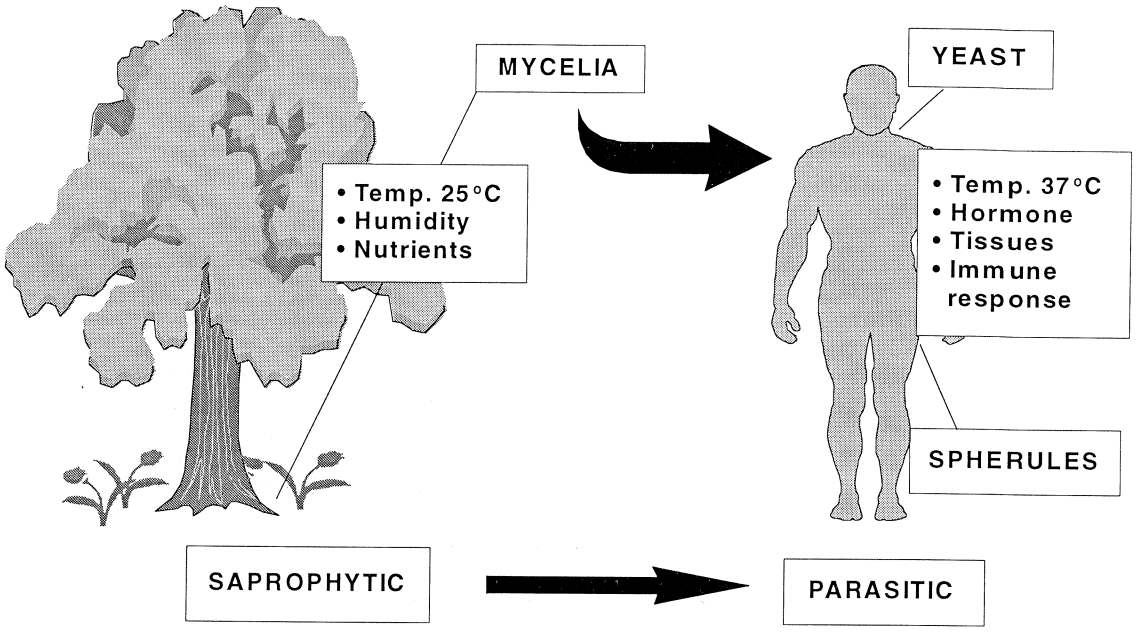

Fig. 1 - Factors that affect the transition from the saprophytic to parasite form in host-fungus relationship.

virulent strains are capable of withstanding drastic temperature changes and of transforming more quickly [72]. Isolates of *S. schenckii* from systemic lesions can grow at 35°C and at 37°C, but isolates from fixed cutaneous lesions can only grow at 35°C [56]. It is believed that even small differences in temperature tolerance can influence the pathogenic potential of a microorganism as well as the form of disease presented by the host [93].

Resistance to temperature changes is also related to the synthesis of heat-shock proteins [48]. Production of these proteins seems to play an important role not only in thermo-adaptation, but also in the mycelium-to-yeast-phase transition in dimorphic fungi [36]. The temperature change from 25°C to 37°C induces a significant synthesis of the heat-shock proteins in *Trypanosoma cruzi* and *Leishmania major* [112]. Studies have correlated thermotolerance of strains of *H. capsulatum* and virulence through the ability to produce heat-shock proteins and the presence of fatty saturated acids in the fungal membrane. Addition of palmitic acid to mycelial cultures of *H. capsulatum* at 25°C increases the transcription of heat-shock proteins mRNAs [67]. Synthesis of these proteins was also verified in strain DY of *P. brasiliensis* by GOLDANI et al. [36]. These authors verified that incubation of mycelial and yeast forms at 37°C increased the synthesis of constitutive proteins in the mycelial form and led to a decrease in yeasts. These findings led to the suggestion that thermal heat-shock proteins may play a role in mycelium-to-yeast-phase transition of *P. brasiliensis*.

Dimorphism

Dimorphism is a fungal characteristic which depends on alteration of temperature and/or nutrients favoring fungal installation and helping the fungus to withstand the aggression by the host. VILLAR et al.[118] observed that dimorphism in *P. brasiliensis* is not always temperature dependent and that nutritional factors may also interfere with this process. This can be detected by adding fetal calf serum to chemically defined and complex culture media, which permit to preserve the phenotypic expression of yeast form at 25°C. Strains of *H. capsulatum* blocked with p-chloromercuricphenylsulfonic acid in the mycelium-to-yeast-phase transition did not initiate infection in mice. These strains can no longer convert to the yeast phase but continue to growth *in vitro* as mycelia even at 37°C [71]. These facts allow to suggest that the ability of transformation to the parasitic form appears to be an important virulence mechanism for the pathogenicity of dimorphic fungi.

In nature, dimorphic fungi frequently occur in their mycelial form. This form induces production of conidia, small propagules capable of establishing in lung tissue [91]. These propagules are infecting forms that are found in *P. brasiliensis*, *B. dermatitidis*, *H. capsulatum* and *C. immitis* [25,35,102]. The size of these propagules may range from 3 to 20 μm in diameter [102]. In some cases, it is believed that a single infecting propagule is sufficient to cause disease, as suggested for coccidioidomycosis [25].

The dimorphism of some pathogenic fungi is related to cell wall components. In *P. brasiliensis* this characteristic feature seems to be closely related to the synthesis of glucan. In the mycelial form there is a predominance of ß-(1,3)-glucan whereas in the yeast form the main polysaccharide is α-(1,3)-glucan [102]. Alpha-(1,3)-glucan was also found in parasitic forms of other fungi such as *B. dermatitidis* and *H. capsulatum*, conferring higher rigidity to the cell wall and resistance to the attack of phagocytes [39,54,102].

The size of the spherules of *C. immitis*, as well as their cell wall composition promote successful parasitism of the fungus. Thus, among mycosis agents, *C. immitis* produces the largest tissue forms that impair the digestion process [25].

Considering the aspects related to dimorphism, it is believed that several factors such as temperature, nutritional factors and those attributed to the host immune response induce the fungus to change its morphology.

Cell wall components and capsule

Both the cell wall and the capsules synthesized by fungi are structures that protect microorganisms from the host attacks (Table 1) and are considered the major targets for studies on virulence [17,38].

Alpha-glucan is a cell wall polysaccharide that has been constantly associated with an increase of virulence in several strains and fungal isolates [101]. Avirulent mutants of *B. dermatitidis* present smaller amounts of α-(1,3)-glucan on the cell wall compared with wild-type and virulent strains of this species. It has been reported that α-glucan seems to mask components of the cell wall in *B. dermatitidis* such as WI-1 antigenic adhesin on the surface of the yeasts and that this adhesin is associated with induction of humoral immune response and macrophage activation [50,52]. Alpha-(1,3)-glucan and ß-(1,3)-glucan are reported to take part in dimorphism and to be involved in virulence aspects of *P. brasiliensis*. Studies carried out on *P. brasiliensis* isolates have suggested that α-(1,3)-glucan protects the fungus against digestive enzymes of the host leukocytes and macrophages [102]. Upon considering the cell wall of *P. brasiliensis* as a virulence factor, SAN-BLAS [101] suggested that human phagocytes may produce ß-glucanase which is capable of digesting only ß-(1,3)-glucan present in cell wall of the mycelial forms of the fungus. Thus, transformation of the fungus into yeast forms at the beginning of infection would prevent the action of phagocytic enzymes on the agent, causing parasitism of *P. brasiliensis*.

Cell wall analysis of the Venezuelan isolate IVIC Pb 9 of *P.brasiliensis* and of mutants derived from this isolate, showed that the amount of α-(1,3)-glucan found in the yeast wall was a potential marker for virulence [102].

SAN-BLAS et al. [99] reported that reduced synthesis of α-(1,3)-glucan on the cell wall of nitrosoguanidine-induced mutants of *P. brasiliensis* resulted in decreased virulence. Other experiments demonstrated that consecutive long-term subculture could also lead to a decrease in α-(1,3)-glucan production. Reversal of this phenomenon may be obtained after fungal culture in medium supplemented with fetal calf serum or after fungus inoculation and recovery from hamsters [100, 102]. However, a study comparing three distinct *P.brasiliensis* isolates (Pb192, Pb18 and Pb265) contradicted this observation and demonstrated that the virulence of *P.brasiliensis* yeast cells was not correlated with the levels of cell wall α-(1,3)-glucan[129].

It was demonstrated that smooth variants of *H. capsulatum* were avirulent and lacked α-(1,3)-glucan on the cell wall compared with rough variants which had this polysaccharide. These rough isolates containing α-(1,3)-glucan were capable of destroying monolayers of macrophages *in vitro*, suggesting an association between α-(1,3)-glucan and the rough isolate virulence [54]. Conversely, EISSENBERG & GOLDMAN [28] observed that some strains of *H. capsulatum* lacking α-glucan on the cell wall were virulent, questioning the role of α-(1,3)-glucan as a virulence factor for *H. capsulatum*. Probably α-(1,3)-glucan acts as a virulence determinant only in some *H. capsulatum* subtypes.

Other cell wall components of *P.brasiliensis* such as ß-glucan stimulate the immune response at higher or lower intensity. An intense immune response would permit lower survival of fungal cells, preventing the host installation and growth. Thus, ß-glucan present on the cell wall of *P. brasiliensis* is capable of inducing a more vigorous inflammatory response and of producing tumor necrosis factor (TNF), an important cytokine which activates the fungicidal activity of macrophages[32, 107]. Regarding *P. brasiliensis*, several authors observed that the low-virulent strain Pb265 induced a higher production of TNF-α and increased chemotaxis for neutrophils compared with the high-virulent strain Pb18, and associated these aspects with large amounts of ß-glucan on the cell wall of low-virulent strains [2, 32, 107].

Among the virulence markers described for *C. neoformans* are the polysaccharide capsule, containing glucuroxylomannan as its major component, and a phenoloxidase enzyme system [59, 94, 121]. It has been postulated that the capsule evolved as a virulence factor in mammals resisting to phagocytes [17]. Several investigators have shown that capsule-deficient mutants occuring naturally or induced by mutagenesis have little or no virulence in mice, compared with encapsulated strains[16, 34, 45, 55]. Production of melanin by *C. neoformans* was reported by STAIB[108] and subsequent studies have demonstrated that this pigment was deposited on the cell wall of the fungus [119].

TABLE 1
Virulence factors associated with the cell wall and capsule of fungi.

Component	*Fungus*	*Activity*
α-(1,3)-glucan	*B. dermatitidis*	Antigenic masking of WR-1 adhesin
	P. brasiliensis	Resistance to digestion by phagocytes.
	H. capsulatum	Destruction of macrophage *in vitro*.
Glucuronoxylomannan	*C. neoformans*	Resistance to phagocytosis
Melanin	*C. neoformans*	Interference with oxidative metabolism of phagocytes.

Melanin is produced from substrates containing dopamine and the action of catalyzing enzymes such as phenoloxidase. It has also been demonstrated that phenoloxidase production is higher at 25°C than at 37°C, suggesting a direct relation between phenoloxidase production and melanin synthesis [42, 44, 121]. Production of melanin-like pigments is a characteristic used for the identification of *C. neoformans* [120, 122] and the ability to produce these pigments has been associated with virulence [59, 94]. *C. neoformans* cells with melanin-like pigments have been observed in human brains [57, 95, 121]. The brain is rich in phenoloxidase substrates such as dopamine, which could help account for the propensity of phenoloxidase-positive organisms to infect the nervous system [120, 130].

Adhesion molecules

Adhesion of pathogenic microorganisms to host tissues has been regarded as the first and major step in colonization and dissemination of the parasite [113].

The cell/cell and cell/extracellular matrix adhesion observed in some fungi such as *P. brasiliensis, B. dermatitidis, H. capsulatum* and *C. neoformans* occurs when the yeast forms have molecules on the cell wall or capsule which permit adhesion and/or dissemination of the fungal cell to other tissues. Fungal adhesion to the host tissues plays a critical role in infection [47, 51]. Bacteria, viruses and fungi use the glycosphingolipids, considered as adhesion receptors present on the cell surface, to bind to host tissues [37, 47, 60, 68]. JIMENEZ-LUCHO et al.[47] observed that yeast forms of *C. neoformans*, *H. capsulatum*, *Candida albicans* and *S. schenckii* bind specifically to lactosylceramide, a glycosphingolipid present in pathogenic cells, suggesting that this molecule was probably responsible for the adhesion of yeasts to host tissues.

P. brasiliensis produces an antigen present on the cell wall, glycoprotein gp43, with the capacity to promote binding to laminin. This molecule is involved in adhesion to the basal membrane or to other components of the extracellular matrix, playing a major role in the dissemination of malignant tumors [65]. VICENTINI et al.[117] infected hamsters with *P. brasiliensis* yeast cells treated with laminin and observed a greater dissemination and severity of the disease. LOPES et al. [65] verified an increase in adhesion of *P. brasiliensis* yeasts to Madin-Darby canine kidney (MDCK) cells. The authors proposed that gp43 would lead to fungus binding to elements of the extracellular matrix, which might explain the dissemination of the fungus in the host from the initial infectious focus [117]. *In vitro* studies demonstrated that gp43 of *P. brasiliensis* is involved in the phagocytosis of this fungus by mouse peritoneal macrophages. Assays of phagocytosis inhibition with D-mannose, D-fucose and D-glucose revealed that gp43 probably binds to macrophages via mannose[1]. Interactions via mannose have been described for other pathogenic fungi [24, 123].

The study of the interaction between macrophage and *P. brasiliensis* is highly significant, since it has been demonstrated that non-activated macrophages allow the growth of the fungus after phagocytosis [10]. Probably, the presence of receptors in host cells favors macrophage-*P. brasiliensis* interaction and macrophage invasion and may stimulate fungal growth within these cells and further dissemination to host tissues.

Other cell cultures have been used to demonstrate that *P. brasiliensis* can bind to and infect cells. Studies of *P. brasiliensis* virulence were conducted by infecting Vero cells cultures from African green monkey kidney, and demonstrated that the fungus presented pathogenicity mechanisms such as adhesion followed by invasion of individual epithelial cells and spread to adjacent cells[74].

It was demonstrated that yeast cells and microconidia of *H. capsulatum* bind to the CD18 family of receptors: CD18/CD11a (LFA1), CD18/CD11b (CR3) and CD18/CD11b (p150,95) present on human monocyte-derived macrophages, alveolar macrophages and PMNs [12, 13, 82]. A protein named WI-1 present on the surface of *B. dermatitidis* yeast cells plays the role of an adhesin and is believed to favor adherence of the fungus to macrophages [51]. KLEIN et al.[50] observed that WI-1 bound to these cells and that avirulent mutants bound more rapidly than the high-virulence wild-type strains. A possible explanation for this fact would be the high-density of WI-1 in avirulent mutants, while in wild type strains this molecule could be masked by the presence of α-(1,3)-glucan [38]. On the other hand, WI-1 seems to be involved in the induction of the host immune response. WI-1 is presented by the macrophages and is bound to the class II molecule of the major histocompatibility complex [53]. In addition, it was observed that WI-1 bound to the CD14 molecule, a receptor for lypopolysaccharide, with a possible involvement in the respiratory burst of macrophages for TNF-α synthesis [124, 125]. MORRISON & STEVENS [76] described an inverse correlation of *in vivo* virulence of *B. dermatitidis* with *in vitro* fungus killing by PMNs and the induction of PMNs superoxide anion production by isolates of *B. dermatitidis* [50].

Thus, in paracoccidioidomycosis, North-American blastomycosis and histoplasmosis, adhesion molecules seem to be associated with the installation, replication and dissemination of the fungus in the host, as well as with the stimulation of the respiratory burst or synthesis of cytokines by the phagocytic cells.

Hormone receptors

Studies using *Saccharomyces cerevisiae* revealed the presence of receptors for 17ß-estradiol in the cytosol of the fungal cell. These high-affinity and high-specificity receptors provided an efficient interaction between the hormones and the receptor. Detailed investigations showed that the fungus has metabolites that bind competitively with 17ß-estradiol binding sites in the yeast and with estrogen receptors, suggesting that hormones may alter the fungal metabolism or that the fungal substances may affect the host metabolism [31].

It was observed that in infections caused by *C. immitis*, more frequent in men than in women, dissemination of the disease was reverted during pregnancy [88]. Studies demonstrated that 17ß-estradiol stimulates the *in vitro* growth of *C. immitis*, altering the rate of spherule maturation and endospore release and that the fungus presents receptor for the hormone in the cytosol [25]. In addition, it

has been reported that other hormones such as testosterone and progesterone also stimulate fungal growth, while some precursors such as ergosterol and cholesterol inhibit *C. immitis* growth [25, 26]. The authors observed that fungi presented receptors for several hormones of the host and that they might influence the pathogenesis of coccidioidomycosis.

The incidence of paracoccidioidomycosis is 13 to 87-fold higher in men than in women. Susceptibility to infection seems to be closely related to hormonal differences between men and women, since contact with *P. brasiliensis* is essentially the same for both sexes [109]. In addition, disease occurs at equal frequency between sexes before puberty. This evidence suggests that the hormonal milieu of the host might influence *P. brasiliensis* pathogenicity [109].

Receptors for 17ß-estradiol were detected in the cytosol of mycelial and yeast forms of *P. brasiliensis*, revealing that this female hormone inhibits mycelium-to-yeast-form transition but does not affect yeast growth or yeast budding [92, 98, 109]. Thus, women's resistance to *P. brasiliensis* infection might be related to the action of estrogens on mycelium-to-yeast-phase transition [109]. Recently, ARISTIZABAL et al.[4] demonstrated that female Balb/c mice intranasally infected with *P. brasiliensis* conidia prevented transformation of these conidia into yeasts. Observations made between 72 and 96 h revealed that males presented decreasing quantities of conidia with a growing increase of yeasts in bronchoalveolar lavage, while in females only conidia were seen. These *in vivo* results confirm the major role of 17ß-estradiol in innate resistance of females to *P. brasiliensis* infection.

DEFAVERI et al.[22], using a murine model, did not notice differences in susceptibility to *P. brasiliensis* infection between males and females. Also, no differences in lesion patterns or in humoral or cellular immune response were detected. However, other authors reported more severe patterns of pulmonary lesions in female mice [70] and higher susceptibility to *P.brasiliensis* infection in female rats [49]. In addition, the study of infection in different phases of the reproductive cycle of DDY female mice demonstrated that female susceptibility was related to the phases in which the estrogen level was low [103].

The controversial results obtained for the susceptibility of males and females to *P. brasiliensis* seem to be due to the use of fungal yeast forms for infection and to the animal species used, since interference of female hormone with the mycelium-to-yeast-phase transition has already been well established.

Enzyme Production

Fungi secrete several hydrolytic enzymes such as proteinases, lipases and phospholipases in culture media. These enzymes, which play a pivotal role in fungal metabolism, may be involved in the pathogenesis of infection, causing damage to the host cells and providing nutrients in a restricted environment [84, 93].

Extracellular proteinases may play a role in adherence and survival of the pathogen on mucosal surfaces [8], invasion of host tissues [83, 97] and digestion of immunoglobulins [97, 127]. Thus, production of proteinases by certain pathogenic fungi has been recognized as a potentially important virulence factor [58, 104].

C. immitis endospores produce proteinases with elastase and collagenase activity. These enzymes were found in culture filtrates of fungus and might play an essential role in the pathogenesis of coccidioidomycosis [90]. A 36kDa alkaline serine-proteinase isolated from supernatants of culture and extracts of *C. immitis* cell wall was capable of digesting human collagen, elastin, hemoglobin and both IgG and secretory IgA [127, 128]. This proteinase, known as Ag11 (antigen 11), is involved in the autolysis and segmentation of mature spherules, a fundamental process for the release of endospores and proliferation of the pathogen [128]. Cleavage of IgG and IgA has been correlated with the ability of yeast colonization and tissue damage, an important process for the initial interaction between parasite and host in the respiratory tract. In addition, release of these proteinases by *C. immitis* parasitic forms in the blood stream may result in interaction of the proteinase with immunoglobulins and compromise the host defense favoring fungus installation and growth [127]. These phenomena suggest that the proteinases are regarded as virulence factors that may favor the pathogenesis of coccidioidomycosis.

Conversely, few studies have examined the potential role of secreted enzymes as virulence factors of *C. neoformans*. This fungus is known for not producing lytic enzymes. However, clinical isolates of *C. neoformans* var. *neoformans* were shown to secrete proteases and extracellular DNase in culture medium [3, 9, 75]. MULLER & SETHI [78] also demonstrated that *C. neoformans* was capable of degrading human plasma proteins.

Production of proteolytic enzymes released in culture media of mycelial and yeast forms of *P. brasiliensis* has been investigated from the same viewpoint. MENDES-GIANINNI et al.[73] verified that a 43 kDa fraction had proteolytic activity on collagen, elastin and casein at pH 6.0 and at 35°C. These results, also demonstrated by other authors [6, 15, 89], might account for fungus evasion of host tissues.

Thus, enzyme production and release by the parasitic phase of pathogenic fungi appear to be involved in the pathogenesis of systemic mycoses, as they are closely related to invasion and tissue damage caused by fungi.

Mechanisms of Evasion from Host Defenses

Pathogenic fungi have several ways to damage vertebrate hosts. Even when the tissue environment is different from their natural habitat, they can survive by adapting their metabolism to higher temperatures and by developing mechanisms to evade host defenses. When facing agressive conditions some fungi are able to use various and complex strategies involving mechanisms such as production of a capsule, utilization of the alternative complement pathway, suppression of cytokine production and reduction of the fungicidal activity of macrophages [38, 115]. These mechanisms lead to immunoregulatory disturbances and impairment of the host defenses.

Immunocompromised patients are the main target of opportunistic infections. Cryptococcosis is usually reported in

patients with impaired cell-mediated immunity, including those with acquired immunodeficiency syndrome, lymphoma, idiopathic CD4 T lymphocytopenia and patients submitted to corticosteroid therapy [27, 63, 86]. Impairment of the host immune system favors the installation of *C. neoformans* through some factors such as the capsule components, which present receptors for C3 component of the complement system [79, 110]. Thus, in cryptococcal sepsis there is a massive activation of the alternative complement pathway, a mechanism used by the fungus to deplete the components of this system and to turn the host more susceptible to infection [66]. Antibodies to glucuronoxylomannan, a capsule component of *C. neoformans*, do not appear to contribute to opsonization of the yeast cells for phagocytosis [40]. Other immunosuppressive effects that have been attributed to capsule components include induction of downregulation of macrophage activity and of antigen presentation [69, 96, 114, 115]. Cryptococcal polysaccharide exert downregulation on human monocytes secretion of stimulatory cytokines such as interleukin-1 and TNF-α [115]. Production of IL-6 and IL-10 by human monocytes stimulated by *C.neoformans* components, suggests a new immunossupressive effect of the fungal antigens on proinflammatory cytokine production by mononuclear phagocytes [23, 64, 116]. In addition to exerting these immunosuppressive effects, fungal antigens may inhibit lymphoproliferation [19] and induce clones of suppressor T cells [7, 46, 96].

The high antigenic load present in the circulation associated with immunosuppression are phenomena observed in coccidioidomycosis[20], histoplasmosis[111] and paracoccidioidomycosis [18].

Modulation of immune response by antigenemia of *P. brasiliensis* was evaluated in an experimental model of paracoccidioidomycosis in hamsters infected intratesticularly. Orchiectomy carried out during the third week of infection increased the animal's survival, prevented depression of cellular immunity and induced a substantial reduction of fungal antigens in serum detected by ELISA [18].

We recently demonstrated (unpublished data) a correlation between antigenemia and suppression indices of cell-mediated immunity in patients with paracoccidioidomycosis. In addition, *in vitro* studies demonstrated that *P. brasiliensis* antigens have a suppressive effect on the lymphocyte proliferative response stimulated with phytohemagglutinin in healthy individuals, suggesting a dose-dependent influence and reproducing the inhibitory effect of the patients' plasmas. Thus, *P. brasiliensis* antigens may play a critical role in the onset of the immunoregulatory disturbances observed in paracoccidioidomycosis.

Stimulation of production of suppressor cells and their related cytokines also seems to be a mechanism used by *P. brasiliensis* to evade host immune response. It was demonstrated in a murine experimental model that intravenous inoculation of *P. brasiliensis* culture filtrate induces the onset of suppressor T cells acting on the delayed type hypersensitivity response [46]. Patients with the most severe forms of paracoccidioidomycosis exhibited high levels of suppressor/cytotoxic T cells and increased Concanavalin A-induced suppressor cell activity [33]. In addition to suppressor T cells, we also detected suppressor activity in culture supernatants of patient monocytes which inhibited production of cytokines and lymphoproliferation of normal lymphocytes. This suppression seems to be due to production of prostaglandins by monocytes [33]. The above-described aspects are supported by observations that patients with paracoccidioidomycosis presented increased numbers of monocytes in peripheral blood [77], a decreased CD4/CD8 ratio [5, 77], inhibition of phagocyte chemotaxis and a low production of IL-2 and its related receptor by patient serum [33,43,80]. High levels of serum antigen may lead to the formation of immunocomplexes which stimulate subpopulations of T cells with suppressor activity or interfere with the activity of the natural killer (NK) cells [87]. Immunocomplexes appear to have an inhibitory effect on NK cells through the interaction with Fc receptor for IgG or by suppressive substances produced by macrophages such as reactive oxygen intermediaries and prostaglandins [11, 85, 106].

Another pivotal mechanism of evasion of host defenses presented by fungi is the interference with the fungicidal activity of phagocytes. *H. capsulatum* is an intracellular parasite that infects macrophages and monocytes after binding to the CD18 class of receptors on these phagocytes [12]. *H. capsulatum* yeasts and microconidia bind to CR3, which is one of the members of the CD18 family, by C3bi-coated fungi particles and fails to elicit an oxidative burst [126]. Thus, *H. capsulatum* needs to avoid exposure to toxic oxigen radicals for successful parasitism, and opsonization has no effect on the ability of organisms to proliferate inside macrophages [41]. The virulence attributed to *H. capsulatum* may be related not only to evasion of the oxidative antimicrobial system but also to its ability to modulate phagolysosomal pH [29]. Following yeast ingestion, phagosome-lysosome fusion occurs. Even so, yeasts multiply intracellularly within the phagolysosome at rates comparable to those observed in *in vitro* culture [38, 41]. These results imply that *H. capsulatum* yeast cells either resist or inactivate the fungicidal activities of lysosomes [41]. These observations suggest that macrophages provide a microenvironment for continued *H. capsulatum* growth and facilitate its dissemination to other tissues.

In addition, pH modulation by *H. capsulatum* yeasts may also influence the amount of intracellular iron available to yeasts within the phagolysosome [38]. Iron is essential for the intracellular survival of *H. capsulatum*, and iron restriction by phagocytes is an important mechanism by which cytokine-activated macrophages kill *H. capsulatum* yeasts[61, 62, 81]. The proliferation of *H. capsulatum* within macrophages terminated with the development of cell-mediated immunity and corresponding activation of macrophages[41]. Only macrophages activated with gamma-interferon may kill *H. capsulatum* by a mechanism involving nitric oxide production[81].

H. capsulatum can use either phagocytes or other cells for its growth and evasion of host tissues. Yeasts may be noted within both alveolar epithelial cells and endothelial cells, suggesting that infected endothelial cells can facilitate the lymphohematogenous spread of the organism [21, 105]. Some *in vitro* experiments have shown that the organisms lacking α-(1,3)-glucan on their cell wall readily entered hamster trachea epithelial cells. These results suggest that non-professional phagocytes can also function as hosts for *H. capsulatum*, promoting its dissemination and evasion [30].

Evasion and virulence mechanisms of different fungi which cause systemic mycoses (Table 2) present multifactorial properties which require the action and interaction of several complex processes. Elucidation of the factors which aid fungi to overcome host defenses will lead to a better understanding of the pathogenesis of systemic mycoses.

TABLE 2
Escape mechanisms of host defenses

Mechanisms	*Fungus*
1- Activation of complement system	*C. neoformans*
2- Intracellular surviving and multiplication	*H. capsulatum* *P. brasiliensis*
3- Downregulation of antigen presentation by macrophage	*C. neoformans*
4- Immunosuppressive effect of fungal antigen on the cytokine production by mononuclear phagocytes	*C. neoformans*
5- Immunosuppression induced by antigenemia	*C. immitis* *H. capsulatum* *P. brasiliensis*
6- Stimulation of suppressor cells	*C. neoformans* *P. brasiliensis*
7- Interference with fungicidal activity of phagocytes	*H. capsulatum*

Final remarks

The literature reviewed here emphasizes the major adaptative mechanisms, also called virulence factors, that allow fungi to survive in a mamalian host. While substantial progress has been made in identifying virulence factors for some fungal pathogens, much work remains to be done to understand the host immune response involved in the pathogenesis of systemic mycoses. The fungal strategies that interphere with the host defense mechanisms are the most interesting and intriguing aspects and actually of great interest to immunologists. The study of these mechanisms will permit a better understanding of the factors involved in the pathogenesis of the mycoses and will also be of great practical importance for the development of effective vaccines against fungal virulence factors. The immunity induced by vaccines must be able in the future to overcome the fungal escape mechanisms and will represent an alternative strategy against establishment of systemic mycoses.

RESUMO

Fatores de virulência em fungos de micoses sistêmicas

Fungos patogênicos causadores de micoses sistêmicas possuem vários fatores que permitem seu crescimento nas condições adversas oferecidas pelo hospedeiro, propiciando o estabelecimento da relação parasitária e contribuindo no processo de doença. Esses fatores são conhecidos como fatores de virulência auxiliando no desenvolvimento da infecção e interferindo com a patogênese das micoses. O presente trabalho avalia os fatores de virulência em fungos patogênicos como *Blastomyces dermatitidis, Coccidioides immitis, Cryptococcus neoformans, Histoplasma capsulatum e Paracoccidioides brasiliensis,* em relação à termotolerância, dimorfismo, componentes da parede celular ou cápsula, bem como a produção de enzimas. Os fatores de virulência auxiliam na aderência, colonização, disseminação e habilidade do fungo para resistir a ambientes hostis e escapar dos mecanismos da resposta imune do hospedeiro.

Tanto os fatores de virulência apresentados por diferentes fungos, como os mecanismos de defesa oferecidos pelo hospedeiro requerem ação e interação de processos complexos, cujo conhecimento permitirá a melhor compreensão da patogenia das micoses sistêmicas.

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