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Virulence Factors of Medically Important Fungi

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INTRODUCTION

Central to a review of virulence factors is the question, "What is a virulence factor?" Strictly and simply defined, it is any factor that a fungus possesses that increases its virulence in the host. Many virulence factors are of such obvious importance that they are often taken for granted. For example, the ability of a fungus to grow at 37°C and physiological pH is a virulence factor for fungi that invade deep tissues and the transition to a parasitic form is essential for the pathogenicity of the dimorphic fungi. Moreover, a size compatible with alveolar deposition is a virulence factor for fungi acquired by inhalation of airborne spores. This review will concentrate on the virulence factors that allow fungi to elude specific aspects of host defenses (Table 1). Virulence factors that affect fungal pathogenicity in nonmammalian hosts (e.g., plant species) will not be discussed. There is strong molecular evidence that *Pneumocystic carinii* is a fungus (154), but both *P. carinii* and *Candida* species have been the subject of several excellent reviews (29–32, 58, 59, 113, 196, 224, 286) and will not be considered in this review.

The ideal test of a virulence factor is to compare biological responses in fungi with and without the factor. Such comparisons have traditionally required the isolation of mutant strains

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TABLE 1. Virulence factors of medically important fungi

Fungal pathogen	Putative virulence factor	Type of mutant	Findings
Aspergillus species[a]			
Proteases	Elastase-serine protease	*N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis	Decreased virulence in immunosuppressed mice
		Gene disruption	No difference in virulence in a murine model of invasive pulmonary aspergillosis
Toxins	Elastase-metalloprotease	NA[b]	
	Aspartic acid proteinase	NA	
	Gliotoxin	NA	
	Restrictocin	Gene disruption	No difference in virulence in a murine model of invasive pulmonary aspergillosis
Other	Aflatoxin	NA	
	Rodlets	Gene disruption	No difference in morbidity in a murine model of invasive pulmonary aspergillosis
	Catalase	NA	
	Lysine biosynthesis	Classical methods	Reduced pathogenicity
	p-Aminobenzoic acid synthesis	Classical methods	Nonpathogenic
Blastomyces dermatitidis[c]	Cell wall α-1,3-glucan	Spontaneous	Decreased α-1,3-glucan content associated with avirulence and rapid clearance from the lungs of mice
	WI-1 adhesin/antigen	Spontaneous	Increased binding of mutants to human macrophage as a result of enhanced expression
Coccidioides immitis[d]	Extracellular proteinases	NA	
	EBP	NA	
Cryptococcus neoformans[a]	Capsule	Spontaneous or mutagenized strains	Reduced virulence in acapsular strains
		Gene disruption of CAP59	Mutant avirulent in mice, restored by complementation with CAP59
	Phenoloxidase/melanin synthesis	UV mutagenesis and classical methods	Lower mortality of Mel$^-$ strains in mice
	Varietal differences	Clinical variants	Var. *gattii* possibly more virulent than var. *neoformans*
	Mating types	Classical methods	α mating type more virulent than **a** mating type
	Adenine biosynthesis (ADE2)	Gene disruption	Avirulent in rabbit chronic meningitis model; virulence restored by ADE2 cDNA
	Mannitol	UV mutagenesis	Low producers killed more readily in vitro by human neutrophils
	Myristoyl-CoA: protein *N*-myristoyltransferase[e]	Targeted gene replacement with temperature-sensitive allelle	Avirulent in an immunosuppressed rabbit model of cryptococcal meningitis
Dematiaceous fungi[d]	Phenoloxidase/melanin synthesis	Spontaneous	Mel$^-$ strains less virulent in lethal infection models
Histoplasma capsulatum[c]	Cell wall α-1,3-glucan	Selection for spontaneous variants	Avirulent in mice, unable to lyse P388D macrophage-like cells in vitro
	Intracellular growth	NA	
	Thermotolerance	Natural variants	Temperature sensitivity associated with reduced virulence
Paracoccidioides brasiliensis[d]	Estrogen-binding proteins	NA	
	Cell wall components		
	β-Glucan	Spontaneous	Increased β-glucan concentrations promote increased TNF levels
	α-1,3-Glucan	Spontaneous	Decreases associated with lowered virulence in mice
	gp43/laminin-binding protein	NA	
Sporothrix schenckii[d]	Thermotolerance	Natural variants	Lowered thermotolerance may restrict dissemination
	Extracellular enzymes	NA	

[a] Transformation system developed and gene disruption available.
[b] NA, not available.
[c] Transformation system developed.
[d] No transformation system available.
[e] CoA, coenzyme A.

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obtained by chemical or UV mutagenesis or by selection of naturally occurring mutants. One major drawback of these approaches is that the mutant strain may be deficient in more than just the factor being studied, either because multiple mutations are present or because the fungi have a general rather than specific defect (e.g., a general defect in protein transport rather than a specific defect in surface expression of a single protein).

Recent advances in fungal molecular biology have begun to make it possible to circumvent these concerns by cloning the genes responsible for producing putative virulence factors and either introducing knockout mutations or expressing the factor in another organism. Wherever possible, our emphasis will be on descriptions of specific gene products that might benefit from a molecular genetic approach, although sometimes it will only be possible to describe a virulence attribute.

The model systems chosen to compare mutants with their parents are critical for the analysis of virulence factors. As an obvious example, an intravenous model of infection would be a poor choice when studying a virulence factor that enables a fungus to survive in the pulmonary compartment after inhalation. Equally important is the readout of the model system. The end result chosen may define pathogenicity as lethality for the host, as alterations in tissue distribution of the organism in the host, as alterations in histopathologic findings, or as alterations in the disease course from an acute disease to an indolent chronic infection.

It is often argued that fungi have evolved for a saprophytic existence and that mammalian infection is not necessary for the survival of any fungal species. Many virulence factors are undoubtedly an "accident of nature" whereby the fungus evolved the factor not to increase its virulence in mammalian hosts but, rather, to increase its survival advantage as a saprophyte or as a pathogen of plants or lower animals. However, other virulence factors, such as thermal dimorphism, have no obvious function in the saprophytic life cycle and appear to have evolved for infection of mammalian hosts. While person-to-person (or mammal-to-mammal) spread is a rare event for most fungi that cause systemic disease, eventually the host will die, and latent or actively replicating fungi can then return to their saprophytic life cycle. Thus, it makes teleological sense that at least some fungal virulence factors have evolved and the simplistic view that fungi are strictly saprobes is probably not valid.

FUNGAL PATHOGENS

Aspergillus Species

Introduction. Aspergillosis refers to the broad range of disease states whose etiologic agents are members of the genus *Aspergillus* (170). Most cases of human disease are caused by *Aspergillus fumigatus*, followed in frequency by *A. flavus* and *A. niger*. Aspergilli are ubiquitous in the environment, and frequent exposure to the fungus via inhalation of airborne conidia (which are an ideal size for alveolar deposition) undoubtedly occurs. Invasive aspergillosis rarely occurs in the immunocompetent host. In the susceptible host, conidia germinate into hyphae, the invasive form of the disease. Most human disease can be divided into three broad categories: allergic aspergillosis, aspergilloma, and invasive aspergillosis. Disparate risk factors are associated with each of the three types of disease. Thus, allergic disease results from an overexuberant immune response in a hypersensitive or atopic individual, whereas invasive disease is usually seen in individuals who are severely immunocompromised as a result of neutropenia, therapy with high doses of corticosteroids, or late-stage AIDS (133, 170).

The wide spectrum of disease states associated with aspergillosis greatly complicates the study of putative virulence factors. For example, a virulence factor associated with invasive aspergillosis might be irrelevant in allergic disease. Moreover, some virulence factors could be important only in association with individual risk factors, such as neutropenia or corticosteroid therapy. Finally, because persons who contract aspergillosis usually have profound disturbances of the immune system, host factors may be more important than fungal virulence factors. Such considerations must be taken into account when designing studies testing candidate virulence factors.

Proteases. *Aspergillus* species secrete a variety of proteases, many of which undoubtedly evolved to allow the fungus to degrade dead animal and vegetable matter for use as nutritional substrates. Whether proteases also function as virulence factors by degrading the structural barriers of the host and thereby facilitating the invasion of host tissues has been the subject of recent intense study (see below), especially with regard to proteases with elastinolytic activity.

Elastin constitutes nearly 30% of lung tissue, and elastinolytic activity has been implicated in the pathogenesis of certain pulmonary bacterial infections (143, 145, 244). Evidence for the possible involvement of elastase in the pathogenesis of aspergillosis was first reported by Kothary et al., who found that 71 of 75 environmental strains of *A. fumigatus* produced elastase (145). The elastase-negative strains were relatively avirulent in a mouse model of invasive pulmonary aspergillosis compared with strains selected for high elastase production. Rhodes et al. extended these findings by demonstrating that all clinical isolates tested from patients with invasive aspergillosis displayed elastinolytic activity in vitro (245). However, examination of tissue blocks from nine patients with invasive pulmonary aspergillosis revealed no evidence of elastolysis (62).

A. fumigatus possesses two elastases, one a serine protease and the other a metalloproteinase, both of which have been cloned and sequenced (143, 193, 200, 202, 203, 266, 269, 280, 281). The serine protease appears to be identical to a previously described 32-kDa alkaline protease (143, 200, 202, 203, 269, 280, 281). Tang et al. created two mutant strains of *A. fumigatus* in which the gene encoding the serine (alkaline) protease was disrupted by molecular biological techniques (280, 281). The mutants had undetectable extracellular elastinolytic activity. The pathogenicity of the mutant strains was similar to that of the parental strains in three murine models of invasive pulmonary aspergillosis (269, 281). Similar results were obtained by Monod et al. (200). In contrast, Kolattukudy et al. created elastase-deficient mutants by exposing *A. fumigatus* to chemical (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) mutagenesis and screening colonies for lack of elastase activity on elastin-containing media (143). Immunosuppressed mice were more susceptible to challenge with the parental strain than with an elastase-deficient mutant. The most likely explanation for the disparate results is that the strain derived by chemical mutagenesis has defects in other functions in addition to elastinolytic activity. However, alternative explanations based on differences in the wild-type strains and/or the murine models of aspergillosis cannot be excluded.

A combination of monoclonal antibodies with inhibitory activity against *A. fumigatus* elastase was tested in two murine models of aspergillosis (98). Antibodies had no effect in an immunosuppressed-mouse model but protected immunocompetent mice given a massive inoculum of conidia. Compared with the serine protease, the role of the *A. fumigatus* metalloproteinase as a putative virulence factor has not been exten-

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sively studied (193, 266). Immunogold electron microscopy revealed that *A. fumigatus* organisms invading neutropenic mouse lungs secrete this metalloprotease (193).

A. flavus also produces elastase, although elastinolytic activity in vitro is considerably lower than that seen with *A. fumigatus* (143). Two elastases have been characterized, a 23-kDa metalloproteinase and a 36-kDa serine proteinase (237, 244). The serine proteinase has been cloned and demonstrates 83% sequence homology to the *A. fumigatus* serine proteinase (237).

In addition to proteases with elastinolytic activity, an aspartic (acid) proteinase has been purified from *A. fumigatus*, and its secretion in vivo was demonstrated by immunofluorescence in the lung of a patient who died of invasive pulmonary aspergillosis (238). Other proteases undoubtedly exist, but the role, if any, that *Aspergillus* proteases play as virulence factors remains uncertain. It may be that a certain degree of redundancy exists, in which case multiple proteases would need to be knocked out to create a strain with reduced or no virulence.

Host defense factors. *A. fumigatus* conidia bind specifically to human fibrinogen as well as to the basement membrane components laminin and fibrinogen (5, 52, 167, 287). Other plasma and basement membrane components, including fibronectin, albumin, and immunoglobulin G, do not bind in appreciable amounts (52). Binding of fibrinogen and laminin was associated with the outer, electron-dense layer of the conidial cell wall and progressively decreased as the conidia germinated (5). The significance of these binding interactions is unclear, but they could be an important first step that allows the fungus to establish residence in host tissues. Interestingly, the protease(s) produced by *A. fumigatus* degrades both fibrinogen and laminin (167, 287). The 33-kDa serine protease appears to be primarily responsible for fibrinogen degradation (167). Thus, fibrinogen and laminin binding could facilitate the adherence of fungal conidia whereas protease production could assist with host cell invasion of the hyphae.

The association of neutropenia and neutrophil dysfunction (in particular, chronic granulomatous disease) has prompted research into neutrophil-*Aspergillus* interactions. While *A. fumigatus* conidia resist killing by neutrophils and neutrophil products, germinating (swollen) conidia and hyphae are readily killed (67, 68, 173, 174, 180, 262). A low-molecular-weight substance present in diffusates from *A. fumigatus* conidia which inhibits the phagocyte respiratory burst as well as the ingestion of conidia bound to the surface of neutrophils and monocytes has been described (250, 251). However, others have shown that following incubation with human neutrophils, virtually all cell-associated *A. fumigatus* conidia are ingested (178). As conidia are adapted to survival in adverse environmental conditions, it is not surprising that they are able to resist neutrophil defenses. However, whether the capacity of *A. fumigatus* conidia to resist neutrophil killing represents a true virulence factor in most clinical situations is debatable, because pulmonary macrophages (rather than neutrophils) have primary contact with the inhaled conidia whereas neutrophils are thought to form the second line of defense should germination occur (180, 262).

The outermost cell wall layer of *Aspergillus* conidia is characterized by the presence of interwoven fascicles of clustered proteinaceous microfibrils called rodlets (284). Rodlets confer hydrophobicity upon *Aspergillus* conidia and are thought to contribute to the efficiency of conidial dispersion in the air. The extreme resistance of rodlets to chemical degradation has suggested a role for them in resistance to phagocytic defenses (284). The gene encoding rodlet protein has been cloned (223, 284), and a rodletless mutant of *A. fumigatus* has been generated (284). The parent strain and the rodletless mutant were similar with respect to their abilities to adhere to type II pneumocytes, fibrinogen, and laminin. Moreover, in a murine model of invasive pulmonary aspergillosis, morbidity was comparable for the two strains (284).

Aspergilli produce catalase, an enzyme that breaks down hydrogen peroxide (H_2O_2) to water and oxygen. The strong association of aspergillosis with chronic granulomatous disease (CGD) (45) provides indirect evidence that catalase is a virulence factor. Neutrophils of CGD patients are unable to mount an effective respiratory burst, and persons with CGD are particularly susceptible to infections with catalase-positive microbes (70, 252). With catalase-negative organisms, neutrophils of CGD patients are able to make up for their deficient H_2O_2 production by utilizing the H_2O_2 produced by the organisms. Catalase-positive organisms, by degrading the H_2O_2 that they produce, deprive the phagocytes of their endogenous H_2O_2. Whether catalase acts as a virulence factor in clinical situations other than CGD remains speculative.

A. fumigatus activates the complement system in normal human serum, with resultant deposition of C3b and C3bi on the surface of the organisms and generation of a potent chemotactic factor (probably C5a) (150, 274, 275, 300). Hyphae activate complement via both the alternative and classical pathways. However, conidia are poor activators of the classical pathway, perhaps a reflection of their poor ability to stimulate antibody production (150). In vitro, *A. fumigatus* and *A. flavus* produce an inhibitor of the alternative pathway of complement (309, 310). Whether clinically significant amounts of the complement inhibitor are produced in clinical infection is unknown.

The increased risk of aspergillosis in persons receiving high doses of corticosteroids is generally thought to be a result of impairment of macrophage and perhaps T-cell function (65, 170, 262, 301). Recently, Ng et al. demonstrated that pharmacological doses of the corticosteroid hydrocortisone modestly enhanced the growth of *A. fumigatus* in culture (218). The clinical significance of this effect requires further study. Of interest is whether *Aspergillus* species have specific steroid-binding proteins analogous to those that have been found on other fungi (93, 186, 187).

Toxins. *A. fumigatus* produces gliotoxin, a metabolite in the epipolythiodioxopiperazine family with a broad range of immunosuppressive properties. Gliotoxin inhibits macrophage phagocytosis (84, 206, 207), as well as T-cell activation and proliferation (206, 278). It induces DNA fragmentation and programmed cell death (apoptosis) in macrophages by mechanisms apparently distinct from its antiphagocytic properties (22, 306, 307). It is not known whether clinically significant amounts of gliotoxin are produced in human disease. In a case report, concentrations of gliotoxin approximately 100 times greater than that needed for in vitro effects were found in a bovine udder infected with *A. fumigatus* (12).

In addition to gliotoxin, *A. fumigatus* produces an 18-kDa cytotoxin that acts by cleaving a phosphodiester bond of the 28S rRNA of eukaryotic ribosomes (10, 222, 268). The literature variably refers to this RNA nuclease as restrictocin, 18-kDa antigen, AspfI, rAspfI/a, and ASPFI (168, 222). Restrictocin is produced in vivo as evidenced by its detection in the urine of patients with invasive aspergillosis and in regions of necrosis surrounding fungal colonies in the kidneys of mice infected with *A. fumigatus* (163, 168, 268). Moreover, restrictocin appears to be a major antigen, as it binds to immunoglobulin E from allergic patients and immunoglobulin G from aspergilloma patients (10). Other medically important species of *Aspergillus* do not make restrictocin (10). To study the role

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of restrictocin in pathogenesis, Paris et al. and Smith et al. independently constructed mutants of *A. fumigatus* by disruption of the gene encoding restrictocin (222, 268). Disruptants were indistinguishable from parental strains in their growth in lung tissue and their pathogenicity in a murine model of invasive pulmonary aspergillosis. Double mutants lacking both restrictocin and extracellular alkaline phosphatase were similar in pathogenicity to wild-type strains in a low-inoculum model of invasive pulmonary aspergillosis (269). These results argue against a significant role for restrictocin in the pathogenicity of invasive aspergillosis. However, because of its immunogenicity, this protein may still play a role in the immune response to *A. fumigatus* (222), especially with regard to the immunopathogenesis of allergic aspergillosis (10).

Aflatoxin, a mycotoxin produced by *A. flavus* and *A. parasiticus*, is an important contaminant of many crops worldwide (90, 249, 314). Aflatoxin is carcinogenic in animal and in vitro models, and consumption of aflatoxin-contaminated feeds has been linked epidemiologically to hepatocellular carcinoma. There is no evidence that aflatoxin acts as a virulence factor in human aspergillosis. An undefined "endotoxin" that is derived from *A. fumigatus* mycelia and is toxic in animal models of aspergillosis has also been described (40).

Miscellaneous factors. An alternative approach to the study of *Aspergillus* virulence factors was provided recently by Mondon et al. (199). These investigators studied the relationship between the ability of *A. fumigatus* strains to invade tissues and genetic polymorphism by using random amplified polymorphic DNA analysis. Of a total of 100 random amplified polymorphic DNA primers, 1 was found to generate a 0.95-kb amplification product that was present in 5 of 9 strains obtained from patients with invasive aspergillosis but only 1 of 13 strains obtained from patients with aspergillomas or bronchial colonization. The gene(s) encoded by the amplification product was not determined. These data need to be verified with more isolates studied prospectively, because the large number of primers studied increases the probability of positive results being obtained by chance alone. However, this study does demonstrate the feasibility of "reverse" approaches in which molecular biology techniques are used as a screening tool to identify potential virulence genes.

To better understand the molecular pathogenesis of medically relevant *Aspergillus* species, a murine model of invasive pulmonary aspergillosis has been established with *A. nidulans* as the inoculum (282). Although human disease is only rarely caused by this organism, the advantages of studying *A. nidulans* include its well-defined genetics and the availability of hundreds of well-characterized mutations (282). Mutants deficient in lysine biosynthesis had reduced pathogenicity, while those deficient in *p*-aminobenzoic acid biosynthesis were nonpathogenic. Supplementing the drinking water of the mice with *p*-aminobenzoic acid restored pathogenicity. A *p*-aminobenzoic acid-requiring mutant of *A. fumigatus* obtained by classical methods also was avirulent in a murine model of aspergillosis (260).

Blastomyces dermatitidis

Blastomyces dermatitidis is a thermally dimorphic fungus that causes a chronic granulomatous and suppurative mycosis of humans and lower mammals (20, 140). The presumed environmental location is the soil, although it is difficult to reproducibly isolate the fungus from nature. The invasive tissue form takes the shape of a broad-based budding yeast. Infection typically presents as an acute or self-limited pneumonia, but chronic pulmonary, cutaneous, and disseminated forms of blastomycosis may occur (3, 140). Areas of endemic infection include the upper midwest, notably northern Wisconsin and Minnesota, and the Mississippi and Ohio River valleys. Work on virulence factors has been limited, but WI-1 antigen and other cell wall constituents are candidates.

Cell wall fractions. The study of virulence factors in *B. dermatitidis* began with the early work of DiSalvo and Denton (72) examining total extractable lipid and phospholipid fractions of whole yeast cells. They correlated increasing lipid content directly with increasing virulence in four strains. No correlation of virulence was seen with phospholipid content. Later, Cox and Best (54) examined a different fraction, trypsin-treated cell walls, from two strains. They associated decreased virulence (strain GA-1) with increased α-1,3-glucan content, and they associated increased virulence (strain KL-1) with increased chitin content and with glucan-containing covalently attached phospholipid. Subsequent studies continue to implicate high phospholipid content of cell walls as a characteristic of highly virulent strains (23, 24).

Evidence for the role of phospholipid in virulence came from intraperitoneal inoculation of mice with cell wall preparations. Cell walls from virulent (KL-1) and avirulent (GA-1) strains elicit different histological reactions, which are hypothesized to be due to different phospholipid contents of the alkali-soluble fraction of trypsin-treated cell walls (55). Trypsin-treated cell walls produce tissue necrosis and death in this assay, but only cell walls from the virulent isolate elicit a characteristic granulomatous response. Fractionation of the cell wall into alkali-soluble and -insoluble fractions separates tissue necrosis and mortality from granulomatous responses. Injection of alkali-soluble fractions (10 to 30 mg) produces a granulomatous response but is not lethal and does not produce necrosis. Only alkali-soluble fractions from the virulent strains produce a granulomatous response at the 10-mg dose. A 30-mg dose is required to see any polymorphonuclear cell (PMN) infiltration when alkali-soluble material from the avirulent strain is injected. The greater ability to produce a granulomatous response correlates with the increased phospholipid component of the alkali-soluble fraction in the virulent strain. Injection of 25 mg of alkali-insoluble cell walls from either strain intraperitoneally is lethal and produces tissue necrosis with only occasional PMN infiltration. This finding suggests that alkali-insoluble fractions contain an endotoxin-like activity.

One difficulty in interpreting previous studies of virulence has been the diverse origin of many standard laboratory strains. Recently, work with a set of related strains was described by Stevens and coworkers which confirms and extends observations made with older isolates. They described two strains spontaneously derived from a highly virulent strain, ATCC 26199, with altered lethality in murine models of infection (23, 24). Attenuated ATCC 60915 arose after in vitro culture, is 10,000-fold less virulent by intranasal challenge, and is cleared from the lung very early after infection (24). Avirulent ATCC 60916 is nonlethal in the same model and is also cleared from the lung within 4 days of infection (201). The wild-type and mutant yeasts differ markedly in their cell wall composition, especially in their expression of α-1,3-glucan and WI-1.

α-1,3-Glucan. Previous studies of *Paracoccidioides brasiliensis* and *Histoplasma capsulatum* have shown an association between loss of the α-1,3-glucan carbohydrate polymer and attenuated virulence of those fungi (103, 141, 259). These observations prompted study of the genetically related strains of *B. dermatitidis* for expression of the polymer. The avirulent mutant (ATCC 60916) has lost all detectable α-1,3-glucan, and

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the attenuated strain (ATCC 60915) has greatly reduced amounts of α-1,3-glucan on the cell surface compared with wild-type ATCC 26199 (111). Although the precise role of the polymer in virulence is unknown for *B. dermatitidis* and other dimorphic fungi, it has been hypothesized that α-1,3-glucan may mask other cell wall components such as the WI-1 adhesin/antigen on the surface of the yeast, at least in *B. dermatitidis* (see below).

WI-1 adhesin/antigen. WI-1 is a 120-kDa cell wall adhesin that has been isolated from the surface of all *B. dermatitidis* strains examined thus far (137). The molecule (110, 136) contains 34 copies of a 25-amino-acid tandem repeat, which are highly homologous to invasin, an adhesin of *Yersinia* spp. The tandem repeats mediate attachment to human monocyte-derived macrophages, mainly through binding complement type 3 (CR3) receptors (110, 215). WI-1 is also a key antigenic target of humoral (136, 137) and cellular (139) responses during human infection; the tandem repeat is the chief site of antibody recognition.

Examination of the related strains ATCC 26199, ATCC 60915, and ATCC 60916 showed that WI-1 expression is significantly altered on the surface of mutant yeasts, along with other changes in protein expression (135). These mutant strains have greatly increased amounts of expressed or exposed WI-1 on the cell wall, and they shed less WI-1 into culture supernatants during in vitro growth. In addition, the mutants bind more avidly to human macrophages through cellular recognition of WI-1. Other work has shown that the mutants also stimulate a respiratory burst that is 20-fold greater than that with the wild-type yeasts upon contact with murine neutrophils (201). It is tempting to speculate that the copious shedding of WI-1 by wild-type yeasts may somehow influence the ability of the virulent strain to escape from recognition and inhibition of replication by phagocytes, as has been described during 72-h cocultures of these yeasts with murine macrophages (see below) (27).

An intriguing feature of the attachment of *B. dermatitidis* yeasts to macrophages is the difference in receptors that bind wild-type and mutant yeasts. Mutant yeasts (and purified WI-1 on latex microspheres) bind CR3 and CD14 receptors on the macrophages, whereas wild-type yeasts bind CR3 alone. CD14 is a receptor for binding lipopolysaccharide alone or complexed to lipopolysaccharide-binding protein (318). Binding CD14 on phagocytes markedly increases the affinity of CR3 receptors for its ligand (317). Thus, the binding of CD14 by WI-1 on mutant yeasts and the cooperative interaction of CD14 and CR3 on the macrophage might help explain the greatly enhanced binding of mutants to the macrophages.

There are two possible explanations for why WI-1 on mutants but not wild-type yeasts mediates attachment to CD14. First, α-1,3-glucan may mask the CD14-binding site of WI-1 on wild-type yeasts whereas a WI-1-binding site for CD14 may be exposed on mutant yeasts devoid of the α-(1,3)-glucan polymer. An alternative explanation is that the high density and possible clustering of WI-1 on the surface of the mutants increase its affinity for CD14 receptors just as these properties enhance the affinity of other ligands for macrophage receptors (106). In contrast, the lower density of WI-1 on wild-type yeasts may have insufficient affinity to bind CD14. The binding of wild-type and mutant yeasts to different receptors on human macrophages, especially the binding of mutant yeasts to CD14 receptors on phagocytes, could influence the inflammatory response to the fungus and may be important for understanding the pathogenesis of blastomycosis.

It is not clear from the foregoing studies of WI-1 how it serves as a virulence factor on wild-type yeasts yet is greatly increased in surface expression and promotes enhanced phagocyte recognition in the hypovirulent mutants. It is hypothesized that WI-1 must be regulated in its expression on the cell surface versus its secretion into the environment in order to achieve its optimal function as a virulence factor. Beachey (13) described good versus bad adherence for the role of *Escherichia coli* and *Proteus mirabilis* pili in the pathogenesis of these gram-negative infections. On mucosal surfaces, the bacteria must possess surface adhesins to adhere. After invading deeper tissues, they must shed their adhesin or produce masking capsules to avoid phagocyte recognition (13). Thus, pathogens must regulate adhesin expression to survive and produce disease in the host.

African blastomycosis. WI-1 is immunologically similar to the previously described A antigen in that both antigens display the 25-amino-acid repeat as an immunodominant B-cell epitope (117, 138). African strains of *B. dermatitidis* have many antigens in common with North American strains, but a distinguishing feature is that African strains lack the A antigen present on most North American strains (131). African strains also tend to cause a distinctive pattern of blastomycosis, which is thought to be less severe and in which chronic cutaneous lesions dominant the clinical picture (163a). This pattern contrasts with the primarily pulmonary and systemic symptoms typically seen with North American strains. It will be interesting to examine the African strains for surface and gene expression of WI-1 and to study whether the presumed loss of expression of WI-1 may be linked with the lower-virulence phenotype and distinct disease pattern. A recently developed transformation system for *B. dermatitidis* (112) will permit direct testing of the role of WI-1 in virulence by using gene complementation to restore WI-1 to the surface of African strains that lack the adhesin.

Coccidioides immitis

Coccidioides immitis is the causative agent of coccidioidomycosis or San Joaquin Valley fever and is endemic to semiarid areas of the southwestern United States and northern Mexico in the Lower Sonoran Life Zone. Over half of infections are asymptomatic (71). Symptomatic infection typically presents as pneumonitis with hilar adenopathy and cutaneous rashes. In its disseminated form, coccidioidomycosis is arguably the most severe of the systemic mycoses, with extensive granulomatous reactions and tissue damage in the skin, bones and joints, meninges, and genitourinary tract. Progression of disease in the immunosuppressed patient is rapid and usually fatal (53).

C. immitis has the most morphologically complex tissue form of the dimorphic fungi. The mycelial form grows in alkaline soils, and alternate cells in the segmented hyphal branches autolyse to release buoyant and highly infectious arthroconidia. The arthroconidia are small (2 to 3 μm by 4 to 5 μm) and easily inhaled into the mammalian lung, where they convert into spherules. The spherules enlarge (20 to 100 μm) and segment internally into hundreds of endospores. Ultimately, mature endospores rupture the spherule cell wall and are released for local and hematogenous dissemination. Each endospore (3 by 4 μm) is capable of developing into a new endosporulating spherule. The sheer size of the endosporulating spherule compared with that in other fungal pathogens, the size of the replicative burst, and the substantial construction of the cell wall contribute to the extreme virulence of the organism (80, 105). Spherules are coated with an extracellular matrix which appears to restrict PMN access (96) and may be responsible for the relative resistance of spherules to PMN-mediated killing (99). Extensive work characterizing extracellular anti-

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gens and proteins with roles in spherule morphogenesis, i.e., chitinase (127) and β-glucanase (152), will not be considered here in the context of virulence factors unless a specific interaction with the host is suggested. Virulence factors that will be described include cell wall-associated proteinases and estradiol-binding proteins. Substantial progress has been made in the cloning and characterization of some of these factors. With the development of a transformation system for *C. immitis*, many important questions can be posed and addressed genetically.

Extracellular proteinases. The extensive dissemination of the organism from the lungs and the profound tissue damage in the lungs and other organs led to the search for fungal extracellular proteinases. In particular, since elastin is a major structural component of both lung interstitium and blood vessels, assays have been designed to detect elastase activity. Numerous investigators searching for proteinases in *C. immitis* have focused upon components of the soluble conidial wall fraction (SCWF) (47). The SCWF is a rich site of humoral and cellular antigens (46, 134) and contains antiphagocytic properties for PMNs (80). It also appears to be immunosuppressive for mouse lymph node cell proliferation (46) and contains several proteolytic enzymes that may participate in morphogenesis and play a role in virulence. Chief among these is a 60-kDa extracellular and cell surface serine proteinase with broad substrate specificity. Proteinase activity against bovine casein is found in mycelial, conidial, and spherule culture filtrates and in cell walls of both conidia and spherules. The proteinase was purified from SCWF fractions and characterized biochemically as a serine proteinase and immunologically as Ag11 by two-dimensional immunoelectrophoresis in a coccidioidin/anti-coccidioidin reference system. Demonstrated substrates include elastin, collagen, and human immunoglobulins G and A. The association of the proteinase with mycelia and conidia suggests that it may be released from outer cell wall of conidia soon after inhalation, leading to immediate damage of respiratory tract tissue (323). Immunoelectron microscopy with rabbit monospecific antiserum against purified proteinase detected the proteinase in the cell walls of mycelia and spherules, in the segmentation apparatus of the developing spherule (324), and in close association with chitin in the presegmentation spherule (48). The density of the proteinase in the segmentation apparatus suggests that one likely role of the proteinase in the fungal life cycle is to promote the dissolution of the wall matrix and segmentation apparatus as the endospores mature. At the same time, the action of the proteinase against human immunoglobulins and against elastin suggests that it may act as a virulence factor by promoting tissue damage when the ruptured spherule releases endospores and residual proteinase. This idea is strengthened by the observation of proteinase associated with the released endospore (324).

The gene encoding this serine proteinase has been cloned and sequenced, and its B-cell epitopes were expressed on a fusion protein encoding a portion of the mature enzyme (48). The open reading frame of the gene predicts a protein of 34.3 kDa, which is in agreement with the 36 kDa observed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. Gel filtration of purified proteinase estimates the molecular mass at 60 kDa (49), most probably representing dimerization. A 5-kDa proteinase inhibitor specific for the serine proteinase has been purified from the mycelial cytosol and endosporulating spherule cell wall. Immunoelectron microscopy shows that it is found in the same locations as the 36-kDa serine proteinase. This inhibitor may function as a regulator of proteolysis during development (322), although this remains speculative.

Cole et al. identified two additional proteinases by substrate gel electrophoresis of crude antigen preparations (49). One proteinase isolated from 5-day-old mycelial culture filtrates cannot degrade immunoglobulins and has a molecular mass of 56 kDa estimated by gel filtration. A second proteinase, AgCS, was isolated from the SCWF as a 19-kDa proteinase, was able to degrade immunoglobulins, and shared many of the attributes of a 23-kDa proteinase isolated by Resnick et al. from spherule culture filtrate (240). The activity of the latter enzyme peaked at 60 h in synchronous in vitro cultures at the time of endospore release. Resnick et al. assayed elastase activity by using intact extracellular matrix secreted by rat vascular smooth muscle (R22) cell lines as a substrate. Most major matrix components including elastin, glycoproteins (fibronectin and laminin), and type I collagen were degraded. An additional proteinase, a metalloproteinase, was also detected in the same study. These investigators postulated a role for proteinases in the release of endospores from the spherule and in local tissue damage. They pointed out that elastin degradation products promote the chemotaxis of inflammatory cells and a damaging inflammatory response (116).

An ideal test of the in vivo role of these proteinases in virulence would be to use nonproteolytic mutants in an animal challenge model. Given the essential role that some of these proteins may play in morphogenesis of the spherule, that may not be feasible. It might be possible to assess the role of proteinases by screening *C. immitis* isolates of differing virulence for quantitative alterations in proteinase expression or for alterations in substrate specificity. Alternatively, model systems could be designed to test the effects of specific proteinase inhibitors upon tissue damage and inflammation.

Estrogen-binding proteins. Estrogen-binding proteins (EBP) in *C. immitis* cytosols may represent virulence factors for the organism. Men are normally four to seven times more likely than women to experience disseminated infection (53). Pregnancy reverses the relative resistance of women to disseminated infection, and the risk of dissemination increases with increasing term. Since the concentrations of numerous hormones rise during pregnancy, hormone effects on *C. immitis* were investigated. Progesterone, testosterone, and 17β-estradiol (E2) stimulate growth in vitro by accelerating the rate of spherule maturation and endospore release. E2, in particular, stimulates growth in a dose-dependent manner and has a striking effect at concentrations consistent with unbound hormone levels during pregnancy (81). High-capacity, low-affinity binding activities specific for progestin, estrogen, androgen, and, to a lesser extent, corticosterone and glucocorticoid hormone classes are found in the cytosol of *C. immitis* (234). Also found are high-affinity, low-capacity binders, one for progestin with a K_d of 1.24 to 36 nM (234) and one for estrogen with a K_d of 21 to 37 nM (233). Both concentrations are consistent with those seen in pregnant women. An androgen binder had a K_d that was too low for interaction with physiological levels of testosterone (233). The multiplicity of hormones with in vitro effects suggest that several human hormones may play a role in modulating *Coccidioides* growth in vivo, but more work is needed to demonstrate any in vivo interactions.

Cryptococcus neoformans

Introduction. Cryptococcosis is a rare disease in hosts without obvious immunocompromise, but its prevalence is greatly increased in persons with impaired cell-mediated immunity (64, 171, 225). Major risk factors for cryptococcosis include AIDS, lymphoma, corticosteroid therapy, and idiopathic CD4 T lymphocytopenia (64, 82, 171, 225). In the United States, in the absence of prophylaxis, about 5 to 10% of persons with

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AIDS contract cryptococcosis at some point in the course of their illness (57, 64, 171). In Africa, this percentage is even higher. Nearly all cases of cryptococcosis are secondary to infection with *Cryptococcus neoformans*; the nearly 20 other cryptococcal species virtually never cause disease in humans (64, 171).

The marked predilection of persons with T-cell dysfunction to cryptococcosis has stimulated intense study of why cell-mediated immunity is of paramount importance to the control of *C. neoformans*. Presumably, other arms of the immune system, including those related to antibody and neutrophil responses, often are inadequate to contain *C. neoformans* in the absence of normal T-cell function. Thus, study of cryptococcal virulence factors has focused not only on the factors that enable *C. neoformans* to elude T-cell defenses but also on the factors that help the organism elude humoral and phagocytic defenses. A comprehensive review of cryptococcosis has recently appeared in this journal (197).

Capsule. *C. neoformans* is the only fungal species of unarguable pathogenicity that has a capsule (14). Cryptococcal capsule is a viscous polysaccharide of which glucuronoxylomannan is the major component. The biochemistry of the capsular polysaccharide (CPS) has recently been reviewed (42). It has been postulated that the capsule evolved not as a virulence factor in mammals but, rather, to enable *C. neoformans* to adapt to changes in environmental conditions. Thus, when nutrients and water are readily available, capsule synthesis is repressed. However, under conditions of decreasing concentrations of moisture or nitrogen, capsule synthesis is stimulated. Moreover, in arid conditions, the hydrophilic capsule collapses and protects the yeast from dehydration (42). The reduced size of *C. neoformans* in the environment is ideal for alveolar deposition following inhalation (213). Regardless of why the capsule evolved, there is unequivocal evidence proving that it is a major virulence factor. Moreover, substantial progress has been made defining the myriad of mechanisms by which the capsule helps *C. neoformans* elude host defenses.

Acapsular (nonencapsulated) strains of *C. neoformans*, obtained either naturally or by mutagenesis, have greatly reduced virulence compared with encapsulated strains (38, 97, 120, 146, 160). Recently, one of the genes, designated CAP59, involved in capsule formation was cloned. Gene deletion by homologous integration resulted in an acapsular phenotype which was avirulent in mice. When a capsule-deficient mutant of *C. neoformans* was complemented with the CAP59 gene, virulence was restored (38). Thus far, these are the only published data of fungal pathogenicity in which the molecular form of Koch's postulates has been met in a nonhousekeeping gene.

In general, *C. neoformans* is thickly encapsulated when observed in mammalian tissues. However, upon culture in artificial media, capsule thickness is variable and strain dependent (101, 184). Physiological concentrations of bicarbonate ion and carbon dioxide (CO_2) markedly stimulate capsule production (101). Moreover, a stable clone that lacked the CO_2-inducible phenotype exhibited small capsules and was avirulent in a rabbit model of crytococcal meningitis (101). Iron deprivation also stimulates capsule synthesis, and the effects of iron deprivation and bicarbonate are additive (292). A reduced capsule size also is found after in vitro culture in media containing superphysiological concentrations of salts or sugars, although that may be at least partially a result of physiochemical contraction of the capsule (124, 292).

A major mechanism by which the capsule seems to enable *C. neoformans* to subvert host defenses is by presenting a surface not recognized by phagocytes (149, 172). Acapsular isolates are readily engulfed by phagocytes, possibly via mannose and β-glucan receptors (56, 147, 181, 247, 267). In contrast, in the absence of opsonins, most phagocyte populations will not bind encapsulated strains of *C. neoformans* (19, 147, 149, 172, 181, 183).

The capsule of *C. neoformans* is a potent activator of the alternative complement pathway (148, 151). Deposition of cleavage fragments of the third component of complement (C3) on the capsular surface serves to opsonize the organisms for immune system recognition by phagocytes (181). However, in cryptococcal sepsis, massive activation of complement by capsular polysaccharide can lead to marked depletion of serum complement components and loss of serum opsonic capacity (188). Moreover, in a mouse model of cryptococcosis, in vivo deposition of C3 on fungal organisms in the brain was essentially absent (288). This observation may help explain the marked propensity of *C. neoformans* to infect the central nervous system, where complement levels are thought to be low (288).

Normal human serum contains antibodies reactive with the glucuronoxylomannan of *C. neoformans* (114). However, such naturally occurring antibodies do not appear to contribute to opsonization of the yeast cells for phagocytosis (114). Circulating CPS not only is poorly immunogenic but also can result in antibody unresponsiveness or tolerance (209, 277). Indeed, antibodies to CPS are often absent in clinical and experimental cryptococcosis (36, 66). In murine models, the antibody response to CPS is highly restricted and only some antibodies are protective (36, 79, 204, 205). Two approaches, one using passive antibody administration and the other using immunization with a glucuronoxylomannan-tetanus toxoid conjugate vaccine, are under development as ways of overcoming the failure of the host to elicit protective antibodies against CPS (63, 326). Some isolates obtained from patients with recurrent cryptococcosis undergo a change in glucuronoxylomannan structure during the course of infection (41). Such a change theoretically could enable the organism to elude antibody defenses.

Whereas acapsular strains of *C. neoformans* are readily phagocytosed by macrophages, the majority of serum-opsonized encapsulated strains remain bound to the surface unless specific anticapsular antibody is added (103, 177, 181). Moreover, most (but not all) phagocyte populations are more efficient at inhibiting and/or killing acapsular than encapsulated strains (175–177, 179, 195). Encapsulated *C. neoformans* strains have been shown to be weak stimulators of the macrophage respiratory burst compared with acapsular strains (172, 175). Other immunosuppressive effects that have been attributed to the presence of capsule or purified CPS include down-regulation of cytokine secretion, inhibition of leukocyte accumulation, induction of suppressor T cells and suppressor factors, inhibition of antigen presentation, and inhibition of lymphoproliferation (15, 25, 51, 78, 179, 182, 208, 211, 295, 296).

Cryptococcosis is one of the most prevalent opportunistic infections in persons with AIDS and generally is associated with a poor prognosis (43). Both purified CPS and whole *C. neoformans* yeast cells have been reported to enhance human immunodeficiency virus type 1 infection and replication in lymphocytes (221, 227, 228). Moreover, human immunodeficiency virus infection of some monocyte, macrophage, and lymphocyte populations diminishes the capacity of these cells to mediate anticryptococcal activity (34, 104).

Phenoloxidase. In 1962, Staib described the development of a brown color by *C. neoformans* when grown on media containing an aqueous extract of *Guizotia abyssinica* birdseeds (229, 271). Subsequent studies have demonstrated that this pigment is due to the deposition of melanin in the cell wall of the fungus. Melanin synthesis is catalyzed by a membrane-

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bound phenoloxidase (laccase) with a substrate specificity for phenolic compounds containing hydroxyl or amino groups, such as L-DOPA and dopamine (161, 230). The enzyme has been purified, and the gene has been cloned (118, 119, 312). While originally described as the basis of diagnostic tests to differentiate *C. neoformans* from other yeasts (64, 171, 229, 271), melanine production also has been implicated as a major virulence factor. Melanin production occurs in vivo and can be demonstrated by using the Masson-Fontana stain for melanin (64, 158).

Isogenic strains apparently differing only in phenoloxidase activity were genetically constructed by classical techniques and studied in a mouse model of cryptococcosis (159, 246). Mortality in mice challenged with melanin-deficient mutants (Mel^-) was strikingly lower than that seen in mice challenged with the parental (Mel^+) strains. About 50% of the isolates obtained from the brains of mice that had died after challenge with Mel^- had reverted to Mel^+ (246). Moreover, the revertants were fully virulent (160, 246). Mel^- isolates also deficient in capsule production were avirulent and did not revert to Mel^+ (160).

Melanins are scavengers of reactive oxygen intermediaries including superoxide anion and singlet oxygen (144). Melanized *C. neoformans* cells are less susceptible than nonmelanized cells to reagent antimicrobial oxidants known or hypothesized to be produced by stimulated phagocytes (125, 231, 303, 305). Thus, one mechanism by which melanin could act as a virulence factor is by making the organisms relatively resistant to leukocyte attack. In support of this hypothesis, melanized *C. neoformans* cells were more resistant to antibody-mediated phagocytosis and the antifungal effects of murine macrophages and a microglial cell line than were nonmelanized organisms (16, 303). Recently, Huffnagle et al. presented evidence of an additional mechanism by which melanin could act as a virulence factor: a high-melanin-producing strain of *C. neoformans* elicited decreased lymphoproliferation and tumor necrosis factor alpha production compared with a strain that produced small amounts of melanin (115).

While the data discussed above provide strong evidence that melanin synthesis is a cryptococcal virulence factor, the specific mutations resulting in the Mel^- phenotype have not been defined. It appears that at least seven genes are involved in melanin production in *C. neoformans* (285). Many of these genes undoubtedly have effects not just on phenoloxidase expression but also on more global functions such as posttranslational modifications (285). Thus, the hypovirulence of Mel^- strains could be secondary not to lack of melanin production but, rather, to defects in other critical yeast cell functions. In support of this theory, UV-induced mutants selected on the basis of hypersensitivity to hyperbaric oxygen also tend to be Mel^- (91, 121). However, revertants which regain the ability to synthesize melanin but still retain their sensitivity to hyperoxia can be isolated (91).

Finally, phenoloxidase activity is depressed at 37°C compared with 25°C (118, 122). This finding, coupled with the finding that melanized *C. neoformans* cells were less susceptible to the fungicidal effects of UV light, suggests that melanin production may have evolved primarily to protect the fungus against ionizing radiation and other adverse environmental conditions (304). Whether phenoloxidase is truly a virulence factor in cryptococcosis should be answered with reasonable certainty once genetically engineered knockout mutations are generated and studied. The association of melanin production and virulence also has been noted for the dematiaceous fungi (see below). 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. Melanin also has been postulated to be a virulence factor in fungi that are primarily plant pathogens (232).

Varietal differences and mating types. *C. neoformans* contains two varieties, *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii*. Each variety contains two serotypes, serotypes A and D for *C. neoformans* var. *neoformans* and serotypes B and C for *C. neoformans* var. *gattii* (162, 171). The two varieties have disparate ecological niches (89, 171). Clinical and host differences also have been noted between infections with the varieties of *C. neoformans* (171, 270). In particular, *C. neoformans* var. *gattii* is more likely than *C. neoformans* var. *neoformans* to infect immunocompetent hosts and to produce neurological sequelae (270). Some experimental data support the notion that *C. neoformans* var. *gattii* is more virulent than *C. neoformans* var. *neoformans*. Binding of alternative complement pathway components and generation of chemotactic factors from human serum appear to be impaired in *C. neoformans* var. *gattii* (78, 308, 321). Moreover, culture filtrate from *C. neoformans* var. *neoformans* stimulated neutrophil migration whereas culture filtrate from *C. neoformans* var. *gattii* inhibited migration (78). However, Kwon-Chung et al. tested the virulence of four serotype B strains of *C. neoformans* var. *gattii* in a mouse model and found the virulence to be similar to that seen with *C. neoformans* var. *neoformans* (162).

C. neoformans occurs in two mating types, α and **a**, which, when crossed on an appropriate medium, fuse to form a basidiomycete, species, *Filobasidiella neoformans* (157). A survey of clinical and environmental isolates revealed that the α type is approximately 30 to 40 times more frequent than the **a** type (155). A pair of congenic *C. neoformans* var. *neoformans* strains with identical phenotypes with regard to colony morphology, growth rate, and expression of phenoloxidase activity but which differed in mating type were constructed. In the parent strains and 10 randomly selected progeny, the α mating type was more virulent than the **a** type (157). While these results link mating type with virulence in *C. neoformans*, the possibility that a virulence-enhancing gene is closely linked to but not part of the mating type locus cannot be excluded (157).

Miscellaneous factors. As with many of the other fungi that cause disease in humans, *C. neoformans* has minimum growth requirements. Thus, host defenses that depend on nutritional deprivation, such as intracellular tryptophan deprivation (as a result of the inhibition of intracellular replication by interferon-induced indoleamine 2,3-dioxygenase activity) (35, 283), are unlikely to be operative in cryptococcosis. *C. neoformans* strains with an inactivated phosphoribosylaminoimidazole carboxylase gene (essential for purine metabolism) were avirulent in a rabbit model of chronic meningitis (226). Virulence was restored in a prototrophic transformant that had received a cloned cDNA copy of the gene. Similar results were obtained when a mutation was introduced into the myristoyl-coenzyme A:protein *N*-myristoyltransferase gene (185).

Compared with studies of other fungi of medical importance, relatively few studies have examined the potential role of secreted enzymes as virulence factors in *C. neoformans*. Extracellular DNase and protease activities have been demonstrated in vitro (6, 198), although their roles as a candidate virulence factors have not been examined. *C. neoformans* appears to have efficient mechanisms for iron acquisition (126, 293), which may be especially advantageous in the iron-depleted milieu of the activated macrophage.

C. neoformans produces and secretes D-mannitol in vitro and in experimental-animal cryptococcosis (219, 313). Mannitol is known to scavenge the hydroxyl radical, a reactive oxygen intermediate generated by neutrophils during the course of a

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respiratory burst. As the oxidative burst is important for optimal neutrophil killing of *C. neoformans* (69), Chaturvedi et al. examined whether secreted mannitol acts as a virulence factor by protecting the fungus against hydroxyl radicals (39). Compared with its parent, a mannitol low-producer strain (derived by classical genetics techniques) was killed significantly more readily by both intact neutrophils and a cell-free hydroxyl radical-generating system.

Dematiaceous Fungi

The dematiaceous fungi include a large group of organisms that are dark walled, in most cases as a result of melanin formation (76). As opposed to melaninogenesis in *C. neoformans*, melanin production in the dematiacious fungi is constitutive and does not require exogenous substrate to produce the Mel^+ phenotype. The diseases produced by the dematiaceous fungi include phaeohyphomycosis, chromoblastomycosis, and black grain mycetoma (76). Although human disease due to dematiaceous fungi is relatively rare, it is usually in the immunocompetent host. Detailed studies have examined the role of melanin as a virulence factor in the dematiaceous dermotropic and neurotropic fungus *Wangiella* (*Exophiala*) *dermatitidis*. Compared with wild-type strains, melanin-deficient (Mel^-) mutants of *W. dermatitidis* show a dramatic increase in the inoculum required to produce acute lethal infection (73–75). However, in a model of chronic infection, a Mel^- strain and its parent both induced similar neurological signs and brain histopathologic abnormalities (75). Compared with Mel^- strains, melanized strains of *W. dermatitidis* were relatively resistant to killing by reagent concentrations of the oxidants permanganate and hypochlorite but not hydrogen peroxide (123).

Histoplasma capsulatum

H. capsulatum is a thermally dimorphic fungus of worldwide distribution, but histoplasmosis is endemic primarily to North and Latin America. Over 95% of infections are either subclinical or mild, self-limited upper respiratory infections. Disseminated disease, while rare, occurs in organs of the reticuloendothelial system and is caused via parasitism of mononuclear phagocytes. Long-term survival within macrophages even after initial disease resolution is responsible for later reactivation when host immunity is compromised (319). Features of avirulent mutants and possible virulence factors related to intracellular growth will be discussed here. Other attributes possibly related to virulence have been comprehensively reviewed by Eissenberg and Goldman (85). Recent achievement of high-level transformation of *H. capsulatum* should allow the role of putative virulence factors to be tested (315, 316).

Avirulent mutants. Klimpel and Goldman demonstrated that α-1,3-glucan-containing cell walls may be a virulence factor (141, 142). Smooth, nonclumping variants of wild-type yeasts that are stable, nonrevertable, and 20- to 100-fold less virulent than the parental isolates were isolated (142). The avirulent mutants also have 1,000-fold less α-1,3-glucan in their cell walls (141). All additional smooth, avirulent mutants isolated from other wild-type strains have lacked α-1,3-glucan (88). Virulent strains can rapidly destroy monolayers of P388D1 macrophage-like cells in vitro, whereas avirulent, smooth derivatives are internalized but cannot multiply intracellularly or lyse the monolayers (88). Conversely, smooth strains can infect hamster tracheal epithelial cell lines with much greater efficiency than do parental, rough strains. Yeasts recovered from rough-strain infections of hamster tracheal epithelial cells are subsequently smooth, α-1,3-glucan deficient, and unable to kill P388D1 macrophage-like cell lines. The observation that <2% of internalized rough yeasts show a smooth phenotype 3 h postinfection and 50% show a smooth phenotype 24 h postinfection suggested that a phenotypic "switch" and not outgrowth of smooth variants has occurred.

The significance of α-1,3-glucan deficiency in these avirulent mutants is unclear, given that *Histoplasma* yeasts naturally occur with two chemotypes (77) differing primarily in α-1,3-glucan content. Chemotype I strains lack α-1,3-glucan, yet several virulent isolates are of this chemotype. Klimpel and Goldman (141) suggested that this discrepancy might be explained by different regulation between the two chemotypes of cell wall growth in vitro. Both this explanation and definite proof of a role for α-1,3-glucan in *Histoplasma* virulence remains to be fully proven.

Intracellular growth. Interaction of *H. capsulatum* and macrophages has been reviewed recently by Newman and Bullock (214). The initial stage of intracellular parasitism by *H. capsulatum* yeasts and microconidia begins with attachment via the CD18 class of receptors to cultured human monocyte-derived macrophages, alveolar macrophages, and PMNs. Phagocytosis occurs rapidly after binding despite variation in the requirements for and rate of binding by each cell type, indicating that binding is the rate-limiting step. *Histoplasma* yeasts are killed in vitro by a combination of H_2O_2, Fe^{2+}, and iodide (214), and ingestion of yeasts by human phagocytes stimulates the respiratory burst, yet intracellular growth of the yeasts is unaffected. Hence, *H. capsulatum* yeasts have evolved mechanisms to survive attack by products of the respiratory burst inside of phagocytes.

A key virulence attribute for *H. capsulatum* may be its ability to modulate phagolysosomal pH. Lysosomal hydrolases require acidic pH for activity, and resistance of the yeasts to phagolysosomal killing by those hydrolases probably stems from elevated pH. Following ingestion of *H. capsulatum* yeasts by mouse P388D1 macrophage-like cell lines (87) or by human macrophages (217), phagosome-lysosome fusion occurs. Even so, *H. capsulatum* yeasts multiply intracellularly within the phagolysosome at rats comparable to those in culture in vitro. The resistance of yeasts to degradation by human macrophages requires both yeast viability and protein synthesis. Heat-killed yeasts or cycloheximide-blocked yeasts are readily eliminated from the phagolysosomes, indicating that macrophage preformed lysosomal hydrolases are sufficient for digestion and that newly synthesized yeast proteins modulate this process. Macrophage digestion of *H. capsulatum* yeasts also requires the maintenance of acidic pH in the phagolysosome, since raising the intracompartmental pH with chloroquine prevents digestion of heat-killed yeasts. Eissenberg et al. (87) measured the ratio of fluorescein excitation at 495 and 450 nm to measure the pH of phagolysosomes containing fluorescein isothiocyanate-labeled yeasts. They showed that live *H. capsulatum* yeasts within mouse P388D1 macrophage-like cell lines maintain elevated pHs of 6.6 in phagolysosomes for up to 5.5 h postinfection. Acridine orange staining suggested that pH may remain neutral for up to 30 hours after infection.

pH modulation by *H. capsulatum* yeasts may also influence the amount of intracellular iron available to yeasts within the phagolysosome. Iron is essential for intracellular survival of *H. capsulatum*, and iron restriction by phagocytes is an important mechanism by which cytokine-activated macrophages kill *H. capsulatum* yeasts (164–166, 212). Newman et al. showed that elevating the pH by chloroquine treatment of infected human monocyte-derived macrophages blocks intracellular replication of the yeasts and promotes macrophage digestion of the ingested yeasts (216). This effect of chloroquine is reversed by the addition of iron nitriloacetate, from which iron

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may be obtained at neutral to alkaline pHs, but not by holotransferrin, which yields iron only at acidic pHs. Thus, modulation of the phagolysosomal pH by *H. capsulatum* yeasts requires a balance between iron availability, requiring an acidic pH for release from transferrin, and inhibition of hydrolase activity, requiring a neutral to alkaline pH. Chloroquine treatment also protects mice from lethal challenge with *H. capsulatum* and significantly reduces the recovery of yeasts from spleen and livers. Such treatment may lead to clinically effective therapy for relapsed histoplasmosis in immunocompromised patients. Nothing is currently known about the mechanism used by *H. capsulatum* yeasts to alter the pH of phagolysosomes. Possible mechanisms include active buffering of the compartment or modification of the ATP-dependent proton pump in the compartment's membrane.

Recent unpublished work has identified gene products specific for intracellular growth. Goldman and coworkers have cloned and sequenced a gene for a calcium-binding protein which is expressed primarily by intracellular yeasts (11, 311). The technique of [^{35}S]methionine labeling of intracellular yeasts followed by SDS-PAGE was used to identify two intracellular-specific proteins of 37.5 and 59 kDa (128), and differential-display reverse transcription-PCR was used to identify over 150 yeast mRNAs associated with growth in murine bone marrow-derived macrophages (190). Nothing is currently known about the function of the last two sets of gene products and their role in intracellular growth.

Thermotolerance. Extensive studies have correlated the relative thermotolerance of *H. capsulatum* strains and virulence. Pathogenicity has been related to the temperature sensitivity of various strains, the level of the heat shock response, and the fatty acid composition of the fungal membrane. That work and the cloning of numerous genes involved in the phase transition are outside the scope of this review. The reader is referred to reviews by Maresca et al. for recent summaries (191, 192).

Agents of Mucormycosis

Mucormycosis (zygomycosis) is the common name given to disease caused by fungi of the class *Zygomycetes*, order *Mucorales* (276). Disease typically results from inhalation of airborne spores and most often manifests itself clinically as sinusitis, rhinocerebral infection, and/or pneumonitis. Like aspergillosis, the pathologic findings are characterized by angioinvasion with resultant hemorrhagic infarction.

The major risk factors for mucormycosis are diabetic ketoacidosis, neutropenia, iron overload, deferoxamine therapy, and protein-caloric malnutrition (60, 276). Mucormycosis is an extremely rare infection in normal hosts, suggesting that fungal virulence factors are operative only when a specific aspect of host defense breaks down. The virulence factors responsible for the susceptibility of persons with these risk factors to mucormycosis has been investigated.

The striking association between the use of the metal chelator deferoxamine in the treatment of iron or aluminum overload and the occurrence of mucormycosis has prompted studies of the mechanisms of iron acquisition by the agents of mucormycosis. In experimental animal models too, pretreatment with deferoxamine shortens survival following challenge with *Rhizopus oryzae* and other agents of mucormycosis (1, 17, 18, 291). Duncan and Artis (83) found that guinea pig serum is naturally fungistatic against *R. oryzae*. However, saturation of serum transferrin by subcutaneous injection of iron-containing compounds partially reversed fungistasis, suggesting that iron availability is critical to the fungistatic capacity of serum.

To explain how deferoxamine, which is an iron chelator, could paradoxically increase susceptibility to mucormycosis, it has been hypothesized that the fungi utilize deferoxamine as a siderophore (291). In support of this hypothesis, in vitro fungal growth and radioiron uptake were stimulated by iron-chelated deferoxamine but not by two iron chelators of the hydroxypyridinone class, L1 (1,2-dimethyl-3-hydroxypiridin-4-one) and CP94 (1,2-diethyl-3-hydroxypyridin-4-one) (18, 297). A 1,000-fold-higher concentration of iron citrate is required to achieve a similar rate of radioiron uptake and in vitro growth stimulation to those observed with iron-chelated deferoxamine (17). Moreover, the efficient ability to utilize the iron in deferoxamine apparently is not shared by *A. fumigatus* or *C. albicans* (17).

Experimental induction of diabetes in mice significantly impairs their ability to withstand intrasinus and pulmonary challenge with *R. oryzae* (2, 299, 302). However, the mechanism of increased susceptibility remains controversial. Waldorf et al. demonstrated that normal but not diabetic bronchoalveolar macrophages inhibited spore germination of *R. oryzae* (301, 302). Serum factors from the diabetic mice prompted both spore germination and impaired attachment of macrophages to spores. The serum factors did not appear to be iron, because neither addition of saturating amounts of exogenous iron nor addition of sufficient transferrin to bind all free serum iron affected spore germination (302). In contrast, Abe found that the diabetic mice that had developed ketoacidosis had lower unbound-iron-binding capacity than did control mice (2). Thus, changes in iron availability to the fungus may play an important role in the pathogenesis of mucormycosis in diabetics with ketoacidosis, but other factors also appear important.

As with the studies with *A. fumigatus* discussed above, human neutrophils and neutrophil products can kill *R. oryzae* hyphae (67, 68, 180, 301). The absence of this host defense in neutropenic patients presumably accounts for the increased susceptibility to mucormycosis.

Paracoccidioides brasiliensis

P. brasiliensis caused a CGD, paracoccidioidomycosis, which is restricted to South and Central America. The precise ecological niche of the mycelial form of this dimorphic fungus remains ill defined but is widely supposed to be the soil of humid tropical and subtropical forests of South America, notably Brazil, Colombia, Venezuela, and Argentina, where paracoccidioidomycosis is endemic. Infection is initiated via inhalation of conidia (194, 243), which convert rapidly to the yeast form in the lungs. Yeasts in tissue frequently have multiple buds forming on a single enlarged mother cell, giving rise to the characteristic "pilot's wheel" appearance. The initial pulmonary disease can disseminate to other organs, including mucous membranes, skin, lymph nodes, and adrenals, although any organ can be affected. Granulomatous responses are often observed in tissues, especially on the face. Early resolution of the disease can leave residual lesions containing viable yeasts, resulting in incapacitating sequelae or relapse up to 40 years later (26, 210, 257, 258). Candidate virulence factors include an estrogen-binding protein, the cell wall constituents α-1,3-glucan and β-glucan, and the extracellular antigen gp43.

Estrogen-binding proteins. Overt paracoccidioidomycosis is 13 to 87 times more common in men than in women (272), despite evidence from skin testing in areas of endemic infection that contact with the fungus is equivalent for both sexes. In addition, disease occurs with equal frequency between the sexes prior to puberty. These facts led to a fruitful series of investigations into the effect of the hormonal milieu of the mammalian host upon *P. brasiliensis* pathogenicity. In 1983,

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Loose et al. demonstrated a high-affinity EBP in the cytosol of *P. brasiliensis* yeasts (K_d = 17 nM) and that 17β-estradiol (E2) inhibited the phase transition from mycelial to yeast forms (187). E2 inhibition is specific for the mycelium-to-yeast transition and does not affect yeast-to-mycelium transition, yeast growth, or yeast budding (241). Subsequent studies reported that both high-affinity (K_d = 6 to 12 nM) and low-affinity (K_d = 150 nM) EPB sites are present in yeast cytosol and that the higher-affinity site is sensitive to temperatures above 37°C (273). This study also reported a higher measured affinity for E2 binding (6 to 12 mM versus 17 nM) than was found previously (187). The new measurements were even closer to physiological concentrations of E2. A slightly lower-affinity-binding (K_d = 13 nM) activity was found in mycelial cytosol (273), as well as a second, much lower-affinity estrogen-binding site. EBP activity in mycelial cytosol also had greater affinity for an estrogen analog, diethylstilbestrol (DES), than did EBP from yeast cytosol, consistent with the low-level inhibition by DES of the mycelium-to-yeast transition (187). Both DES and E2 also inhibit the conidium-to-yeast transition (254). In all cases, DES is only 1 to 2% as potent as E2, and other hormones show no activity in EBP binding or inhibition of phase transition. Inhibition of transition by E2 is mediated in part by altered protein synthesis and in part by altered methionine utilization (44). Recent reviews (26, 210, 257, 258) contain detailed descriptions of these studies and of the current working hypothesis that circulating E2 hormone in adult women act to delay or inhibit the transition of the infecting particle (conidial or mycelial fragment) to the parasitic yeast form.

Currently, the fungal ligand for the EBP remains unknown, as does the function of the hormone receptor-ligand complex in the life cycle of the yeast. We are unaware if any effort has been made to correlate altered expression of EBP or altered binding characteristics between strains with levels of virulence or with disease progression. Some variation in EBP-measured affinities was seen in one study (241), but the differences were small and the patient prognosis associated with each of the various isolates was not presented. Despite this uncertainty, EBP acts as a "negative" virulence factor, since its presence seems to so profoundly alter the outcome of infection in the relatively resistant adult female population. It should be noted that animal models have failed to consistently replicate this disease imbalance in females (132, 261), and further research will be needed to understand the divergence of the model systems from clinical findings.

Cell wall components. The role of the cell wall constituents α-1,3-glucan and β-1,3-glucan in the regulation of the dimorphic transition in *P. brasiliensis* has been studied for many years and has been the subject of recent reviews (257, 258). Wild-type yeast cells contain almost exclusively α-1,3-glucan, and many mutants are available for study (255, 256, 259). A lowered α-1,3-glucan content in the cell wall of the yeast form has been frequently correlated with lowered virulence (103, 259), although some avirulent mutants retain wild-type levels of α-1,3-glucan (129, 325). The importance of α-1,3-glucan as a virulence factor is suggested mainly by indirect evidence. Multiple observations have shown that extended in vitro culture of virulent *P. brasiliensis* isolates inevitably leads to lowered cell wall α-1,3-glucan levels, thinner cell walls as measured by electron microscopy, and loss of virulence in various animal models (28, 37, 130, 259). All three characteristics are regained either by passage through animals or by growth in vitro with fetal calf serum (28, 130). The history of extended subculture of many clinical isolates has led to questioning of older studies of virulence in which the culture history was not outlined explicitly. α-1,3-Glucan is found on the outer periphery of the cell wall, and that location has led to frequent speculation that this substance may play a protective role against host defenses.

Another role of α-1,3-glucan may be in its relationship to β-glucan. Lowered α-1,3-glucan content may serve to unmask β-glucan in the cell wall or may alter the relative proportions of the polysaccharides. Evidence for the role of β-glucan as a virulence factor is much more direct. β-Glucan has been implicated as an important immunomodulator by Silva and coworkers who investigated the role of cell wall fractions in initiation of the host inflammatory response (reviewed in references 21 and 242). In human as well as animal models of infection, infiltrates of PMNs and mononuclear cells are usually found in initial inflammatory sites (21, 242) and also in chronic granulomatous lesions (95). Intraperitoneal injections into mice of alkali-insoluble fractions containing β-glucan (F1 cell wall fractions) result in death, and autopsy reveals wasting with peritonitis and inflammatory exudate (264). Upon intravenous injection of the same fraction, histopathologic examination of lung and spleen tissues shows intense and focal infiltrates of PMNs by day 4, followed by mononuclear cells typically organized and forming epithelioid-type cells. Intraperitoneal injections into rats of the F1 cell wall fractions were used to demonstrate that the resulting peritoneal infiltrate is composed initially of a massive PMN migration peaking at 4 to 8 h and is superseded by mononuclear-cell migration peaking at 48 h (4).

The role of β-glucan as an immunomodulator is further illustrated by differential effects of cell wall fractions prepared from avirulent and virulent isolates. The F1 fraction from avirulent strain Pb265 injected intraperitoneally into rats induces a greater inflammatory cell influx than does the F1 fraction from a recent clinical isolate, PbHC (263). Intraperitoneal injection of purified β-glucan from both strains induces a cellular influx in the same dose-dependent manner. Additionally, subcutaneous injection of F1 fractions into mice results in the formation of nodular granulomas. Granulomatous lesions arising from avirulent Pb265 F1 fraction are more intense and resolve more quickly than do those resulting from the PbHC fraction. The active component from both preparations is not chitin but β-glucan, which is twofold more abundant in the avirulent Pb265 strain. Injections of the F1 fractions intraperitoneally into mice result in elevation of tumor necrosis factor (TNF) levels in serum (94), an observation mimicking the elevated levels of circulating TNF seen in patients (265). F1 fractions from the avirulent isolate (Pb265) induce higher levels of TNF in serum than do those from a virulent isolate, Pb18, because of the higher β-glucan content of the former. Injection of purified β-glucan from each strain gives an identical dose-response curve of TNF induction. Finally, peritoneal macrophages from uninfected mice secrete TNF in vitro in response to dead fungus, F1 cell wall fraction, or purified β-glucan. It is evident that β-glucan as well as other components of the cell wall play roles in modulating host cytokines and the characteristic inflammatory response seen clinically in paracoccidioidomycosis. The details of how β-glucan regulates the host cytokine response, including TNF, and the effect of these cytokines upon granuloma formation remain to be discovered.

Laminin binding. The strongest candidate as a virulence factor in *P. brasiliensis* is gp43. A cell surface and exocellular glycoprotein with proteinase activity at acidic pH (235, 236) that is recognized by 100% of patient sera tested by immunoblotting (33), gp43 acts as a laminin receptor and may be responsible for the adhesion of *P. brasiliensis* yeasts to the basement membrane. Laminin binding to purified gp43 is specific and saturable, and binding to yeast cells in the same nanomolar range can also be demonstrated. gp43 may also be

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responsible for invasion of host cells expressing laminin receptors by laminin-coated yeasts (298). Evidence for the latter role comes from two lines of experiments. Pretreatment of yeasts with 10 or 20 μg of laminin per ml increases in vitro adhesion to Madin-Darby canine kidney (MDCK) cells measured quantitatively and confirmed by scanning electron microscopy. Second, pretreatment of yeasts with laminin before injection into hamster testicles enhances the granulomatous response and increases pathogenicity in an in vivo model. The suggestion has been made that secretion of gp43 may serve to trap host antibody responses and allow cell surface gp34 to bind laminin, promoting dissemination and invasion of host tissues (298). Initially, it was found that gp43 has gelatinase activity that varies among different isolates. However, this activity does not correlate with isolate virulence, and its role in pathogenesis remains obscure (294). The epitopes for gp43 have been cloned and expressed in *Escherichia coli* (279). The availability of this gene will theoretically make it possible to examine the role of gp43 by molecular genetic techniques as they are developed for *P. brasiliensis.*

Sporothrix schenckii

Sporothrix schenckii is found widely distributed in the environment, growing on plant debris in the soil and the bark of trees and shrubs (156). This fungus is the only dimorphic fungal pathogen that does not typically cause a systemic disease. Instead, infection by *S. schenckii* is initiated by traumatic implantation into the skin of mycelia or conidia from the soil. At body temperature, yeasts arise from conidia, and infection typically results in suppurative and granulomatous cutaneous nodules spreading indolently along local lymphatic channels. Extracutaneous sporotrichosis and hematogenous dissemination are much rarer, as is primary pulmonary sporotrichosis (248). Sporotrichosis is endemic worldwide, and focal outbreaks have been reported in North American (50). Little is known about factors contributing to the virulence of this organism, but some candidate factors have emerged from investigations of thermotolerance and extracellular enzymes and polysaccharides.

Thermotolerance. Clinical isolates of *S. schenckii* from fixed cutaneous lesions (nonlymphangitic) are frequently able to grow at 35 but not 37°C in vitro. Kwon-Chung inoculated mice intraperitoneally with a variety of isolates and reported that *S. schenckii* yeasts unable to grow at temperatures above 35°C are restricted to those that cause infection of skin and testes (153). Older studies involving intracardiac and intraperitoneal inoculation of young male rate demonstrated that the lesions are more pronounced when rats are held at lower ambient temperatures (189), also suggesting a connection between thermotolerance and virulence in extracutaneous tissue. The factor(s) responsible for thermotolerance is unknown. It should be noted that one group of investigators reported being unable to replicate Kwon-Chung's observations about thermotolerance in an examination of 20 additional disease-causing isolates (61). Specifically, two isolates from disseminated arm ulcers were unable to grow at 37°C. However, insufficient clinical data were presented to support or refute the classification of those two cases as disseminated disease as opposed to multiple lesions resulting from autoinoculation. All other isolates, whether from fixed cutaneous or disseminated disease, could grow at both 35 and 37°C. Clinically, local thermotherapy has an excellent therapeutic effect and has been found to increase the PMN killing rate in short-term assays (108).

Extracellular enzymes. Acid phosphatases are hypothesized to play a role in the interaction of *S. schenckii* yeasts with macrophages, although there is still no direct evidence for such a role. Acid phosphatase activity is produced by conidia, mycelia, and yeast forms of *S. schenckii*, with the largest amounts of activity associated with yeast extracts (8). Growth temperature (8) and cell shape (7) regulate multiple electrophoretically distinct isoenzymes. Cytochemical studies localized acid phosphatases to vacuoles, the inner cell envelope, and periplasmic sites and extracytoplasmic microfibrillar zones (100). An extracytoplasmic phosphatase activity, Y1, is largely associated with yeast extracts and is the principal activity associated with the microfibrillar zone of yeast cells (9). This acid phosphatase in the microfibrillar zone is hypothesized to influence the interaction of *S. schenckii* yeasts with macrophages and other host cells (100) by analogy with *Leishmania donovani* acid phosphatase interactions with neutrophils and macrophages (239). Treatment of human neutrophils with *L. donovani* acid phosphatase in vitro inhibits superoxide production by modification of neutrophil surface receptors.

The direct interaction of *S. schenckii* with macrophages was shown in a model of chronic sporotrichosis in which testicular nodules form after intraperitoneal inoculation of mice. These nodules contain clusters of PMNs and macrophages with ingested fungi. Yeasts remain viable in these nodules at the end of 6 months (109) despite being primarily inside of the macrophages. It is unknown which yeast factor(s) may be responsible for the long-term survival of yeasts in such chronic infection. One in vitro study suggested that fungal acid phosphatase is not active within the phagolysosome (253). After ingestion of yeasts by murine bone marrow-derived macrophages, fungal acid phosphatase (optimum pH 5.0) was seen in small cytoplasmic granules rather than in association with the fungal cell envelope as previously observed by Garrison and Arnold (100). The activity of the macrophage acid phosphatase (optimum pH 7.2) was associated with the phagosome membrane and with partially degraded fungal cells. Since it may be difficult to compare the observations from a long-term in vivo model with those from an in vitro model, the significance of this discrepancy between studies is uncertain. Other factors influencing macrophage function include cell surface polysaccharides. In vitro phagocytosis of yeasts by peritoneal macrophage is inhibited by galactomannan and rhamnomannans purified from *S. schenckii* cell surfaces (220).

The ability of *S. schenckii* to invade the skin and cutaneous tissues led to investigations of extracellular proteinases as tissue-invading factors. Two distinct extracellular proteinases are produced when *S. schenckii* is grown in media containing albumin or collagen as a nitrogen source (290). Proteinase I is a 36.5-kDa serine proteinase, inhibited by chymostatin, with an optimal pH of 6.0. Proteinase II is a 39-kDa aspartic proteinase, inhibited by pepstatin, with an optimal pH of 3.5. Altering the pH of the culture medium can be used to force the production of one or the other proteinase, but the cell growth rate is unaffected. Likewise, chymostatin or pepstatin alone does not affect cell growth, but the addition of both inhibitors at 10 μg/ml each strongly inhibits replication in vitro (289). Purified proteinase I or II hydrolyzes human stratum corneum, type I collagen, and elastin, all natural components of the skin. Hard keratin (human nails) and elastin are only slightly digested, and type IV collagen is not a substrate. Proof of the in vivo expression of these two proteinases came from studies involving intracutaneous inoculation of hairless mice for a model of infection and spontaneous healing. High-titer antibody responses to both proteinases as measured by enzyme-linked immunosorbent assay develop during the first week and remain high until resolution and healing of the infected nodules at the fifth and sixth weeks (320). Treatment of murine lesions with the pro-

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teinase inhibitors chymostatin and pepstatin at 0.1% each in a topical ointment strongly suppresses nodule formation and leads to faster regression. Either inhibitor alone at 0.1% only slightly suppresses the disease process (169). This model system is not entirely satisfactory because of the spontaneous healing of infected control mice during the course of the treatment, yet it clearly demonstrates an in vivo role of these two proteinases in fungal growth.

CONCLUSIONS

The frequent and rapid emergence of "new" fungal pathogens and the reemergence of "old" pathogens makes it imperative that the mechanisms of virulence be understood. For instance, in the past 10 years, the dimorphic fungus *Penicillium marneffei* has come to light as an organism endemic to Southeast Asia that is capable of causing systemic disease in both healthy and immunocompromised host (107). Despite its "new" identity as an AIDS-defining infection of HIV-positive patients, nothing is known about aspects of the pathogenicity of this organism. While substantial progress has been made in identifying virulence factors for some fungal pathogens, much work remains to be done for some of the more genetically intractable fungi, especially the dimorphic pathogens. Advances in molecular genetics for the dimorphic fungal pathogens have come only recently, and of those organisms, only *H. capsulatum* can be transformed readily enough to test virulence factors. Such tests have proved invaluable for the analysis of putative virulence factors from more genetically malleable organisms such as *Aspergillus* spp. and *C. neoformans*. Falkow has discussed the application of molecular Koch's postulates for the assessment of virulence factors and their genes (92) from bacterial pathogens, and these principles can be extended to studies of fungi. It was the goal of this review to assess the success of similar analyses of fungal pathogens when technology permitted and to illustrate candidate virulence factors in need of rigorous genetic testing. Rapid progress in molecular genetics will allow the development of alternative strategies for analysis of the virulence of these organisms and the development of better treatments for the diseases they cause.

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REFERENCES

1. **Abe, F., H. Inaba, T. Katoh, and M. Hotchi.** 1990. Effects of iron and desferrioxamine on *Rhizopus* infection. Mycopathologia **110:**87–91.
2. **Abe, F., H. Shibuya, M. Tateyama, Y. Ommura, N. Azumi, and K. Kimura.** 1986. Mucormycosis in diabetic ketoacidosis. Role of unbound iron binding capacity of transferrin. Acta Pathol. Jpn. **36:**1507–1512.
3. **Al-Doory, Y., and A. F. DiSalvo (ed.).** 1992. Blastomycosis. Plenum Medical Book Co., New York.
4. **Alves, L. M., F. Figueiredo, S. L. Brandao Filho, I. Tincani, and C. L. Silva.** 1987. The role of fractions from Paracoccidioides brasiliensis in the genesis of inflammatory response. Mycopathologia **97:**3–7.
5. **Annaix, V., J. P. Bouchara, G. Larcher, D. Chabasse, and G. Tronchin.** 1992. Specific binding of human fibrinogen fragment D to *Aspergillus fumigatus* conidia. Infect. Immun. **60:**1747–1755.
6. **Aoki, S., S. Ito-Kuwa, K. Nakamura, J. Kato, K. Ninomiya, and V. Vidotto.** 1994. Extracellular proteolytic activity of *Cryptococcus neoformans*. Mycopathologia **128:**143–150.
7. **Arnold, W. N., L. C. Mann, and R. G. Garrison.** 1987. Media-induced departures from the usual, temperature-dependent cell shapes of *Sporothrix schenckii* and concomitant changes in the acid phosphatase isoenzyme patterns. Microbios **52:**161–171.
8. **Arnold, W. N., L. C. Mann, K. H. Sakai, R. G. Garrison, and P. D. Coleman.** 1986. Acid phosphatases of *Sporothrix schenckii*. J. Gen. Microbiol. **132:** 3421–3432.
9. **Arnold, W. N., K. H. Sakai, and L. C. Mann.** 1987. Selective inactivation of an extra-cytoplasmic acid phosphatase of yeast-like cells of *Sporothrix schenckii* by sodium fluoride. J. Gen. Microbiol. **133:**1503–1509.
10. **Arruda, L. K., B. J. Mann, and M. D. Chapman.** 1992. Selective expression of a major allergen and cytotoxin, Asp f I, in *Aspergillus fumigatus*. Implications for the immunopathogenesis of *Aspergillus*-related diseases. J. Immunol. **149:**3354–3359.
11. **Batanghari, J. W., and W. E. Goldman.** 1995. Cloning and expression characteristics of a gene encoding a calcium-binding protein from *Histoplasma capsulatum*, abstr. F-67, p. 98. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
12. **Bauer, J., M. Gareis, A. Bott, and B. Gedek.** 1989. Isolation of a mycotoxin (gliotoxin) from a bovine udder infected with *Aspergillus fumigatus*. J. Med. Vet. Mycol. **27:**45–50.
13. **Beachey, E. H.** 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143:**325–345.
14. **Bhattacharjee, A. K., J. E. Bennett, and C. P. J. Glaudemans.** 1984. Capsular polysaccharides of *Cryptococcus neoformans*. Rev. Infect. Dis. **6:**619–624.
15. **Blackstock, R., J. M. McCormack, and N. K. Hall.** 1987. Induction of a macrophage-suppressive lymphokine by soluble cryptococcal antigens and its association with models of immunologic tolerance. Infect. Immun. **55:** 233–239.
16. **Blasi, E., R. Barluzzi, R. Mazzolla, B. Tancini, S. Saleppico, M. Puliti, L. Pitzurra, and F. Bistoni.** 1995. Role of nitric oxide and melanogenesis in the accomplishment of anticryptococcal activity by the BV-2 microglial cell line. J. Neuroimmunol. **58:**111–116.
17. **Boelaert, J. R., M. de Locht, J. Van Cutsem, V. Kerrels, B. Cantinieaux, A. Verdonck, H. W. Van Landuyt, and Y. J. Schneider.** 1993. Mucormycosis during deferoxamine therapy is a siderophore-mediated infection. In vitro and in vivo animal studies. J. Clin. Invest. **91:**1979–1986.
18. **Boelaert, J. R., J. Van Cutsem, M. de Locht, Y. J. Schneider, and R. R. Crichton.** 1994. Deferoxamine augments growth and pathogenicity of *Rhizopus*, while hydroxypyridinone chelators have no effect. Kidney Int. **45:** 667–671.
19. **Bolanos, B., and T. G. Mitchell.** 1989. Phagocytosis and killing of *Cryptococcus neoformans* by rat alveolar macrophages in the absence of serum. J. Leukocyte Biol. **46:**521–528.
20. **Bradsher, R. S.** 1992. Blastomycosis. Clin. Infect. Dis. **14:**S82–S90.
21. **Bradsher, R. W., and R. W. McDonnell.** 1994. *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis*, p. 217–237. *In* H. Chmel, M. Bendinelli, and H. Friedman (ed.), Pulmonary infections and immunity. Plenum Press, New York.
22. **Braithwaite, A. W., R. D. Eichner, P. Waring, and A. Mullbacher.** 1987. The immunomodulating agent gliotoxin causes genomic DNA fragmentation. Mol. Immunol. **24:**47–55.
23. **Brass, C., C. M. Volkmann, H. P. Klein, C. J. Halde, R. W. R. Archibald, and D. A. Stevens.** 1982. Pathogen factors and host factors in murine pulmonary blastomycosis. Mycopathologia **78:**129–140.
24. **Brass, C., C. M. Volkmann, D. E. Philpott, H. P. Klein, C. J. Halde, and D. A. Stevens.** 1982. Spontaneous mutant of *Blastomyces dermatitidis* attenuated in virulence for mice. Sabouraudia **20:**145–158.
25. **Breen, J. F., I. C. Lee, F. R. Vogel, and H. Friedman.** 1982. Cryptococcal capsular polysaccharide-induced modulation of murine immune responses. Infect. Immun. **36:**47–51.
26. **Brummer, E., E. Castaneda, and A. Restrepo.** 1993. Paracoccidioidomycosis: an update. Clin. Microbiol. Rev. **6:**89–117.
27. **Brummer, E., P. A. Morozumi, D. E. Philpott, and D. A. Stevens.** 1981. Virulence of fungi: correlation of virulence of *Blastomyces dermatitidis* in vivo with escape from macrophage inhibition of replication in vitro. Infect. Immun. **32:**864–871.
28. **Brummer, E., A. Restrepo, L. H. Hanson, and D. A. Stevens.** 1990. Virulence of *Paracoccidioides brasiliensis*: the influence of *in vitro* passage and storage. Mycopathologia **109:**13–17.
29. **Calderone, R.** 1994. Molecular pathogenesis of fungal infections. Trends Microbiol. **2:**461–463.
30. **Calderone, R., and J. Sturtevant.** 1994. Macrophage interactions with *Candida*. Immunol. Ser. **60:**505–515.
31. **Calderone, R. A.** 1993. Recognition between *Candida albicans* and host cells. Trends Microbiol. **1:**55–58.
32. **Calderone, R. A., and P. C. Braun.** 1991. Adherence and receptor relationships of *Candida albicans*. Microbiol. Rev. **55:**1–20.
33. **Camargo, Z. P., C. Unterkircher, and L. R. Travassos.** 1989. Identification of antigenic polypeptides of *Paracoccidioides brasiliensis* by immunoblot-

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ting. J. Med. Vet. Mycol. **27:**407–412.
34. **Cameron, M. L., D. L. Granger, T. J. Matthews, and J. B. Weinberg.** 1994. Human immunodeficiency virus (HIV)-infected human blood monocytes and peritoneal macrophages have reduced anticryptococcal activity whereas HIV-infected alveolar macrophages retain normal activity. J. Infect. Dis. **170:**60–67.
35. **Carlin, J. M., and J. B. Weller.** 1995. Potentiation of interferon-mediated inhibition of *Chlamydia* infection by interleukin-1 in human macrophage cultures. Infect. Immun. **63:**1870–1875.
36. **Casadevall, A., and M. D. Scharff.** 1991. The mouse antibody response to infection with *Cryptococcus neoformans*: VH and VL usage in polysaccharide binding antibodies. J. Exp. Med. **174:**151–160.
37. **Castaneda, E., E. Brummer, D. Pappagianis, and D. A. Stevens.** 1987. Chronic pulmonary and disseminated paracoccidioidomycosis in mice: quantitation of progression and chronicity. J. Med. Vet. Mycol. **25:**377–387.
38. **Chang, Y. C., and K. J. Kwon-Chung.** 1994. Complementation of a capsule-deficient mutation of *Cryptococcus neoformans* restores its virulence. Mol. Cell. Biol. **14:**4912–4919.
39. **Chaturvedi, V., S. L. Newman, and B. Wong.** 1995. Oxidative killing of *Cryptococcus neoformans* by human neutrophils: evidence that fungal mannitol protects by scavenging hydroxyl radicals, p. 102. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
40. **Chaudhary, B., and B. Singh.** 1983. Role of endotoxin of *Aspergillus fumigatus* in its pathogenicity. Mykosen **26:**430–434.
41. **Cherniak, R., L. C. Morris, T. Belay, E. D. Spitzer, and A. Casadevall.** 1995. Variation in the structure of glucuronoxylomannan in isolates from patients with recurrent cryptococcal meningitis. Infect. Immun. **63:**1899–1905.
42. **Cherniak, R., and J. B. Sundstrom.** 1994. Polysaccharide antigens of the capsule of *Cryptococcus neoformans*. Infect. Immun. **62:**1507–1512.
43. **Chuck, S. L., and M. A. Sande.** 1989. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. N. Engl. J. Med. **321:** 794–799.
44. **Clemons, K. V., D. Feldman, and D. A. Stevens.** 1989. Influence of oestradiol on protein expression and methionine utilization during morphogenesis of *Paracoccidioides brasiliensis*. J. Gen. Microbiol. **135:**1607–1617.
45. **Cohen, M. S., R. E. Isturiz, H. L. Malech, R. K. Root, C. M. Wilfert, L. Gutman, and R. H. Buckley.** 1981. Fungal infection in chronic granulomatous disease. The importance of the phagocyte in defense against fungi. Am. J. Med. **71:**59–66.
46. **Cole, G. T., T. N. Kirkland, and S. H. Sun.** 1987. An immunoreactive, water-soluble conidial wall fraction of *Coccidioides immitis*. Infect. Immun. **55:**657–667.
47. **Cole, G. T., L. M. Pope, M. Huppert, S. H. Sun, and P. Starr.** 1985. Wall composition of different cell types of *Coccidioides immitis*, p. 112–129. *In* H. E. Einstein and A. Catanzaro (ed.), Coccidioidomycosis. Proceedings of the 4th International Conference. The National Foundation for Infectious Diseases, Washington, D.C.
48. **Cole, G. T., S. Zhu, L. Hsu, D. Kruse, K. R. Seshan, and F. Wang.** 1992. Isolation and expression of a gene which encodes a wall-associated proteinase of *Coccidioides immitis*. Infect. Immun. **60:**416–427.
49. **Cole, G. T., S. Zhu, S. Pan, L. Yuan, D. Kruse, and S. H. Sun.** 1989. Isolation of antigens with proteolytic activity from *Coccidioides immitis*. Infect. Immun. **57:**1524–1534.
50. **Coles, F. B., A. Schuchat, J. R. Hibbs, S. F. Kondracki, I. F. Salkin, D. M. Dixon, H. G. Chang, R. A. Duncan, N. J. Hurd, and D. L. Morse.** 1992. A multistate outbreak of sporotrichosis associated with sphagnum moss. Am. J. Epidemiol. **136:**475–487.
51. **Collins, H. L., and G. J. Bancroft.** 1991. Encapsulation of *Cryptococcus neoformans* impairs antigen-specific T-cell responses. Infect. Immun. **59:** 3883–3888.
52. **Coulot, P., J. P. Bouchara, G. Renier, V. Annaix, C. Planchenault, G. Tronchin, and D. Chabasse.** 1994. Specific interaction of *Aspergillus fumigatus* with fibrinogen and its role in cell adhesion. Infect. Immun. **62:**2169–2177.
53. **Cox, R. A.** 1993. Coccidioidomycosis, p. 173–212. *In* J. W. Murphy, H. Friedman, and M. Bendinelli (ed.), Fungal infections and immune responses. Plenum Press, New York.
54. **Cox, R. A., and G. K. Best.** 1972. Cell wall composition of two strains of *Blastomyces dermatitidis* exhibiting differences in virulence for mice. Infect. Immun. **5:**449–453.
55. **Cox, R. A., L. R. Mills, G. K. Best, and J. F. Denton.** 1974. Histologic reactions to cell walls of an avirulent and a virulent strain of *Blastomyces dermatitidis*. J. Infect. Dis. **129:**179–186.
56. **Cross, C. E., and G. J. Bancroft.** 1995. Ingestion of acapsular *Cryptococcus neoformans* occurs via mannose and beta-glucan receptors, resulting in cytokine production and increased phagocytosis of the encapsulated form. Infect. Immun. **63:**2604–2611.
57. **Currie, B. P., and A. Casadevall.** 1994. Estimation of the prevalence of cryptococcal infection among patients infected with the human immunodeficiency virus in New York City. Clin. Infect. Dis. **19:**1029–1033.
58. **Cutler, J. E.** 1991. Putative virulence factors of *Candida albicans*. Annu. Rev. Microbiol. **45:**187–218.
59. **Cutler, J. E., and T. Kanbe.** 1993. Antigenic variability of *Candida albicans* cell surface. Curr. Top. Med. Mycol. **5:**27–47.
60. **Daly, A. L., L. A. Velazquez, S. F. Bradley, and C. A. Kauffman.** 1989. Mucormycosis: association with deferoxamine therapy. Am. J. Med. **87:** 468–471.
61. **de Albornoz, M. B., M. Mendoza, and E. D. de Torres.** 1986. Growth temperatures of isolates of *Sporothrix schenckii* from disseminated and fixed cutaneous lesions of sporotrichosis. Mycopathologia **95:**81–83.
62. **Denning, D. W., P. N. Ward, L. E. Fenelon, and E. W. Benbow.** 1992. Lack of vessel wall elastolysis in human invasive pulmonary aspergillosis. Infect. Immun. **60:**5153–5156.
63. **Devi, S. J. N., R. Schneerson, W. Egan, T. J. Ulrich, D. Bryla, J. B. Robbins, and J. E. Bennett.** 1991. *Cryptococcus neoformans* serotype A glucuronoxylomannan-protein conjugate vaccines: synthesis, characterization, and immunogenicity. Infect. Immun. **59:**3700–3707.
64. **Diamond, R. D.** 1995. *Cryptococcus neoformans*, p. 2331–2340. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's principles and practice of infectious diseases. Churchill Livingstone Inc., New York.
65. **Diamond, R. D.** 1983. Inhibition of monocyte-mediated damage to fungal hyphae by steroid hormones. J. Infect. Dis. **147:**160.
66. **Diamond, R. D., and J. E. Bennett.** 1974. Prognostic factors in cryptococcal meningitis: a study in 111 cases. Ann. Intern. Med. **80:**176–181.
67. **Diamond, R. D., and R. A. Clark.** 1982. Damage to *Aspergillus fumigatus* and *Rhizopus oryzae* hyphae by oxidative and nonoxidative microbicidal products of human neutrophils in vitro. Infect. Immun. **38:**487–495.
68. **Diamond, R. D., R. K. Krzesicki, B. Epstein, and W. Jao.** 1978. Damage to hyphal forms of fungi by human leukocytes *in vitro*: a possible host defense mechanism in aspergillosis and mucormycosis. Am. J. Pathol. **91:**313–324.
69. **Diamond, R. D., R. Root, and J. E. Bennett.** 1972. Factors influencing killing of *Cryptococcus neoformans* by human leukocytes in vitro. J. Infect. Dis. **125:**367–375.
70. **Dinauer, M. C.** 1993. The respiratory burst oxidase and the molecular genetics of chronic granulomatous disease. Crit. Rev. Clin. Lab. Sci. **30:** 329–369.
71. **DiSalvo, A. F. (ed.).** 1983. Occupational mycoses. Lea & Febiger, Philadelphia.
72. **DiSalvo, A. F., and J. F. Denton.** 1963. Lipid content of four strains of *Blastomyces dermatitidis* of different mouse virulence. J. Bacteriol. **85:**927–931.
73. **Dixon, D. M., J. Migliozzi, C. R. Cooper, Jr., O. Solis, B. Breslin, and P. J. Szaniszlo.** 1992. Melanized and non-melanized multicellular form mutants of *Wangiella dermatitidis* in mice: mortality and histopathology studies. Mycoses **35:**17–21.
74. **Dixon, D. M., A. Polak, and G. W. Conner.** 1989. Mel− mutants of *Wangiella dermatitidis* in mice: evaluation of multiple mouse and fungal strains. J. Med. Vet. Mycol. **27:**335–341.
75. **Dixon, D. M., A. Polak, and P. J. Szaniszlo.** 1987. Pathogenicity and virulence of wild-type and melanin-deficient *Wangiella dermatitidis*. J. Med. Vet. Mycol. **25:**97–106.
76. **Dixon, D. M., and A. Polak-Wyss.** 1991. The medically important dematiaceous fungi and their identification. Mycoses **34:**1–18.
77. **Domer, J. E.** 1971. Monosaccharide and chitin content of cell walls of *Histoplasma capsulatum* and *Blastomyces dermatitidis*. J. Bacteriol. **107:**870–877.
78. **Dong, Z. M., and J. W. Murphy.** 1995. Effects of the two varieties of *Cryptococcus neoformans* cells and culture filtrate antigens on neutrophil locomotion. Infect. Immun. **63:**2632–2644.
79. **Dromer, F., J. Charreire, A. Contrepois, C. Carbon, and P. Yeni.** 1987. Protection of mice against experimental cryptococcosis by anti-*Cryptococcus neoformans* monoclonal antibody. Infect. Immun. **55:**749–752.
80. **Drutz, D. J., and M. Huppert.** 1983. Coccidioidomycosis: factors affecting the host-parasite interaction. J. Infect. Dis. **147:**372–390.
81. **Drutz, D. J., M. Huppert, S. H. Sun, and W. L. McGuire.** 1981. Human sex hormones stimulate the growth and maturation of *Coccidioides immitis*. Infect. Immun. **32:**897–907.
82. **Duncan, R. A., C. F. von Reyn, G. M. Alliegro, Z. Toossi, A. M. Sugar, and S. M. Levitz.** 1993. Idiopathic CD4+ T-lymphocytopenia—four patients with opportunistic infections and no evidence of HIV infection. N. Engl. J. Med. **328:**393–398.
83. **Duncan, R. L., Jr., and W. M. Artis.** 1982. Fungistatic capacity of sera from guinea pigs injected with various iron solutions: differences between *Trichophyton mentagrophytes* and *Rhizopus oryzae*. Infect. Immun. **35:**368–370.
84. **Eichner, R. D., M. Al Salami, P. R. Wood, and A. Mullbacher.** 1986. The effect of gliotoxin upon macrophage function. Int. J. Immunopharmacol. **8:**789–797.
85. **Eissenberg, L. G., and W. E. Goldman.** 1991. *Histoplasma* variation and adaptive strategies for parasitism: new perspectives of histoplasmosis. Clin. Microbiol. Rev. **4:**411–421.
86. **Eissenberg, L. G., W. E. Goldman, and P. H. Schlesinger.** 1993. His-

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toplasma capsulatum modulates the acidification of phagolysosomes. J. Exp. Med. **177:**1605–1611.
87. **Eissenberg, L. G., P. H. Schlesinger, and W. E. Goldman.** 1988. Phagosome-lysosome fusion in P388D1 macrophages infected with *Histoplasma capsulatum*. J. Leukocyte Biol. **43:**483–491.
88. **Eissenberg, L. G., J. L. West, J. P. Woods, and W. E. Goldman.** 1991. Infection of P388D1 macrophages and respiratory epithelial cells by *Histoplasma capsulatum*: selection of avirulent variants and their potential role in persistent histoplasmosis. Infect. Immun. **59:**1639–1646.
89. **Ellis, D. H., and T. J. Pfeiffer.** 1990. Ecology, life cycle, and infectious propagule of *Cryptococcus neoformans*. Lancet **336:**923–925.
90. **Ellis, W. O., J. P. Smith, B. K. Simpson, and J. H. Oldham.** 1991. Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and methods of control. Crit. Rev. Food Sci. Nutr. **30:**403–439.
91. **Emery, H. S., C. P. Shelburne, J. P. Bowman, P. G. Fallon, C. A. Schulz, and E. S. Jacobson.** 1994. Genetic study of oxygen resistance and melanization in *Cryptococcus neoformans*. Infect. Immun. **62:**5694–5697.
92. **Falkow, S.** 1988. Molecular Koch's postulates applied to microbial pathogenicity. Rev. Infect. Dis. **10:**S274–S276.
93. **Feldman, D., Y. Do, A. Burshell, P. Stathis, and D. S. Loose.** 1982. An estrogen-binding protein and endogenous ligand in *Saccharomyces cerevisiae*: possible hormone receptor system. Science **218:**297–298.
94. **Figueiredo, F., L. M. Alves, and C. L. Silva.** 1993. Tumour necrosis factor production in vivo and in vitro in response to Paracoccidioides brasiliensis and the cell wall fractions thereof. Clin. Exp. Immunol. **93:**189–194.
95. **Figueiredo, F., C. L. Silva, L. M. C. Alves, and M. A. Rossi.** 1986. Participation of *Paracoccidioides brasiliensis* lipids and polysaccharides in the evolution of granulomas. Braz. J. Med. Biol. Res. **19:**651A.
96. **Frey, C. L., and D. J. Drutz.** 1986. Influence of fungal surface components on the interaction of Coccidioides immitis with polymorphonuclear neutrophils. J. Infect. Dis. **153:**933–943.
97. **Fromtling, R. A., H. J. Shadomy, and E. S. Jacobson.** 1982. Decreased virulence in stable, acapsular mutants of *Cryptococcus neoformans*. Mycopathologia **79:**23–29.
98. **Frosco, M. B., T. Chase, Jr., and J. D. Macmillan.** 1994. The effect of elastase-specific monoclonal and polyclonal antibodies on the virulence of *Aspergillus fumigatus* in immunocompromised mice. Mycopathologia **125:** 65–76.
99. **Galgiani, J. N.** 1986. Inhibition of different phases of Coccidioides immitis by human neutrophils or hydrogen peroxide. J. Infect. Dis. **153:**217–222.
100. **Garrison, R. G., and W. N. Arnold.** 1983. Cytochemical localization of acid phosphatases in the dimorphic fungus *Sporothrix schenckii*. Curr. Microbiol. **9:**253–258.
101. **Granger, D. L., J. R. Perfect, and D. T. Durack.** 1985. Virulence of *Cryptococcus neoformans*. Regulation of capsule synthesis by carbon dioxide. J. Clin. Invest. **76:**508–516.
102. **Griffin, F. M.** 1981. Roles of macrophages Fc and C3b receptors in phagocytosis of immunologically coated Cryptococcus neoformans. Proc. Natl. Acad. Sci. USA **78:**3853–3857.
103. **Hallak, J., F. San-Blas, and B. San-Blas.** 1982. Isolation and wall analysis of dimorphic mutants of *Paracoccidioides brasiliensis*. Sabouraudia **20:**51–62.
104. **Harrison, T. S., H. Kornfeld, and S. M. Levitz.** 1995. The effect of infection with human immunodeficiency virus on the anticryptococcal activity of lymphocytes and monocytes. J. Infect. Dis. **172:**665–671.
105. **Hector, R. F., and D. Pappagianis.** 1982. Enzymatic degradation of the walls of spherules of *Coccidioides immitis*. Exp. Mycol. **6:**136–152.
106. **Hermanowski-Vosatka, A., P. A. Detmers, O. Gotze, S. C. Silverstein, and S. D. Wright.** 1988. Clustering of ligand on the surface of a particle enhances adhesion to receptor-bearing cells. J. Biol. Chem. **263:**17822.
107. **Hilmarsdottir, I., J. L. Meynard, O. Rogeaux, G. Guermonprez, A. Datry, C. Katlama, G. Brücker, A. Coutellier, M. Danis, and M. Gentilini.** 1993. Disseminated *Penicillium marneffei* infection associated with human immunodeficiency virus: a report of two cases and a review of 35 published cases. Acquired Immune Defic. Syndr. **6:**466–471.
108. **Hiruma, M., and S. Kagawa.** 1986. Effects of hyperthermia on phagocytosis and intracellular killing of *Sporothrix schenckii* by polymorphonuclear leukocytes. Mycopathologia **95:**93–100.
109. **Hiruma, M., K. Yamaji, T. Shimizu, H. Ohata, and A. Kukita.** 1988. Ultrastructural study of tissue reaction of mice against *Sporothrix schenckii* infection. Arch. Dermatol. Res. **280:**S94–S100.
110. **Hogan, L. H., S. Josvai, and B. S. Klein.** 1995. Genomic cloning, characterization, and functional analysis of the major surface adhesin WI-1 on *Blastomyces dermatitidis* yeasts. J. Biol. Chem. **270:**30725–30732.
111. **Hogan, L. H., and B. S. Klein.** 1994. Altered expression of surface α-1,3-glucan in genetically related strains of *Blastomyces dermatitidis* that differ in virulence. Infect. Immun. **62:**3543–3546.
112. **Hogan, L. H., and B. S. Klein.** Transforming DNA integrates at multiple sites in the dimorphic fungal pathogen *Blastomyces dermatitides*. Gene, in press.
113. **Hostetter, M. K.** 1994. Adhesins and ligands involved in the interaction of *Candida* spp. with epithelial and endothelial surfaces. Clin. Microbiol. Rev. **7:**29–42.
114. **Houpt, D. C., G. S. T. Pfrommer, B. J. Young, T. A. Larson, and T. R. Kozel.** 1994. Occurrences, immunoglobulin classes, and biological activities of antibodies in normal human serum that are reactive with *Cryptococcus neoformans* glucuronoxylomannan. Infect. Immun. **62:**2857–2864.
115. **Huffnagle, G. B., G.-H. Chen, J. L. Curtis, R. A. McDonald, R. M. Strieter, and G. B. Toews.** 1995. Down-regulation of the afferent phase of T cell-mediated pulmonary inflammation and immunity by a high melanin-producing strain of *Cryptococcus neoformans*. J. Immunol. **155:**3507–3516.
116. **Hunninghake, G. W., J. M. Davidson, S. Rennard, S. Szapiel, J. E. Gadek, and R. G. Crystal.** 1981. Elastin fragments attract macrophage precursors to diseased sites in pulmonary emphysema. Science **212:**925–927.
117. **Hurst, S. F., and L. Kaufman.** 1992. Western immunoblot analysis and serologic characterization of *Blastomyces dermatitidis* yeast form extracellular antigen. J. Clin. Microbiol. **30:**3043–3049.
118. **Ikeda, R., and E. S. Jacobson.** 1992. Heterogeneity of phenol oxidases in *Cryptococcus neoformans*. Infect. Immun. **60:**3552–3555.
119. **Ikeda, R., T. Shinoda, T. Morita, and E. S. Jacobson.** 1993. Characterization of a phenol oxidase from *Cryptococcus neoformans* var. *neoformans*. Microbiol. Immunol. **37:**759–764.
120. **Jacobson, E. S., D. J. Ayers, A. C. Harrell, and C. C. Nicholas.** 1982. Genetic and phenotypic characterization of capsule mutants of *Cryptococcus neoformans*. J. Bacteriol. **150:**1292–1296.
121. **Jacobson, E. S., and H. S. Emery.** 1991. Catecholamine uptake, melanization, and oxygen toxicity in *Cryptococcus neoformans*. J. Bacteriol. **173:**401–403.
122. **Jacobson, E. S., and H. S. Emery.** 1991. Temperature regulation of the cryptococcal phenoloxidase. J. Med. Vet. Mycol. **29:**121–124.
123. **Jacobson, E. S., E. Hove, and H. S. Emery.** 1995. Antioxidant function of melanin in black fungi. Infect. Immun. **63:**4944–4945.
124. **Jacobson, E. S., M. J. Tingler, and P. L. Quynn.** 1989. Effect of hypertonic solutes upon the polysaccharide capsule in *Cryptococcus neoformans*. Mycoses **32:**14–23.
125. **Jacobson, E. S., and S. B. Tinnell.** 1993. Antioxidant function of fungal melanin. J. Bacteriol. **175:**7102–7104.
126. **Jacobson, E. S., and S. E. Vartivarian.** 1992. Iron assimilation in *Cryptococcus neoformans*. J. Med. Vet. Mycol. **30:**443–450.
127. **Johnson, S. M., and D. Pappagianis.** 1992. The coccidioidal complement fixation and immunodiffusion-complement fixation antigen is a chitinase. Infect. Immun. **60:**2588–92.
128. **Kamei, K., E. Brummer, K. Clemons, and D. A. Stevens.** 1992. De novo protein synthesis by *Histoplasma capsulatum* (Hc) after ingestion by macrophages (M), abstr. F-41, p. 505. *In* Abstracts of the 92nd General Meeting of the American Society for Microbiology 1992. American Society for Microbiology, Washington, D.C.
129. **Kanetsuna, F.** 1981. Ultrastructural studies on the dimorphism of *Paracoccidioides brasiliensis*, *Blastomyces dermatitidis* and *Histoplasma capsulatum*. Sabouraudia **19:**275–286.
130. **Kashino, S. S., L. M. Singer-Vermes, V. L. Calich, and E. Burger.** 1990. Alterations in the pathogenicity of one Paracoccidioides brasiliensis isolate do not correlate with its in vitro growth. Mycopathologia **111:**173–180.
131. **Kaufman, L., P. G. Standard, R. J. Weeks, and A. A. Padhye.** 1983. Detection of two *Blastomyces dermatitidis* serotypes by exoantigen analysis. J. Clin. Microbiol. **18:**110–114.
132. **Kerr, I. B., G. V. Schaeffer, and D. S. Miranda.** 1984. Sex hormones and susceptibility of the rat to paracoccidioidomycosis. Mycopathologia **88:**149–154.
133. **Khoo, S. H., and D. W. Denning.** 1994. Invasive aspergillosis in patients with AIDS. Clin. Infect. Dis **19**(Suppl. 1)**:**S41–S48.
134. **Kirkland, T. N., S. Zhu, D. Kruse, L. Hsu, K. R. Seshan, and G. T. Cole.** 1991. *Coccidioides immitis* fractions which are antigenic for immune T lymphocytes. Infect. Immun. **59:**3952–3961.
135. **Klein, B. S., S. Chaturvedi, L. H. Hogan, J. M. Jones, and S. L. Newman.** 1994. Altered expression of the surface protein WI-1 in genetically-related strains of *Blastomyces dermatitidis* that differ in virulence regulates recognition of yeasts by human macrophages. Infect. Immun. **62:**3536–3542.
136. **Klein, B. S., L. H. Hogan, and J. M. Jones.** 1993. Immunological recognition of a 25-amino acid repeat arrayed in tandem on a major antigen of *Blastomyces dermatitidis*. J. Clin. Invest. **92:**330–337.
137. **Klein, B. S., and J. M. Jones.** 1990. Isolation, purification and radiolabeling of a novel 120-kD surface protein on *Blastomyces dermatitidis* yeasts to detect antibody in infected patients. J. Clin. Invest. **85:**152–161.
138. **Klein, B. S., and J. M. Jones.** 1994. Purification and characterization of the major antigen WI-1 from *Blastomyces dermatitidis* yeasts and immunological comparison with A antigen. Infect. Immun. **62:**3890–3900.
139. **Klein, B. S., P. M. Sondel, and J. M. Jones.** 1992. WI-1, a novel 120-kilodalton surface protein on *Blastomyces dermatitidis* yeast cells, is a target antigen of cell-mediated immunity in human blastomycosis. Infect. Immun. **60:**4291–4300.
140. **Klein, B. S., J. M. Vergeront, and J. P. Davis.** 1986. Epidemiologic aspects

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of blastomycosis, the enigmatics systemic mycosis. Semin. Respir. Infect. **1:**29–39.
141. **Klimpel, K. R., and W. E. Goldman.** 1988. Cell walls from avirulent variants of *Histoplasma capsulatum* lack α-(1,3)-glucan. Infect. Immun. **56:**2997–3000.
142. **Klimpel, K. R., and W. E. Goldman.** 1987. Isolation and characterization of spontaneous avirulent variants of *Histoplasma capsulatum*. Infect. Immun. **55:**528–533.
143. **Kolattukudy, P. E., J. D. Lee, L. M. Rogers, P. Zimmerman, S. Ceselski, B. Fox, B. Stein, and E. A. Copelan.** 1993. Evidence for possible involvement of an elastolytic serine protease in aspergillosis. Infect. Immun. **61:**2357–2368.
144. **Korytowski, W., B. Kalyanaraman, I. A. Menon, T. Sarna, and R. C. Sealy.** 1986. Reaction of superoxide anions with melanins: electron spin resonance and spin trapping studies. Biochim. Biophys. Acta **882:**145–153.
145. **Kothary, M. H., T. Chase, Jr., and J. D. Macmillan.** 1984. Correlation of elastase production by some strains of *Aspergillus fumigatus* with ability to cause pulmonary invasive aspergillosis in mice. Infect. Immun. **43:**320–325.
146. **Kozel, T. R., and J. Cazin, Jr.** 1971. Nonencapsulated variant of *Cryptococcus neoformans*. I. Virulence studies and characterization of soluble polysaccharide. Infect. Immun. **3:**287–294.
147. **Kozel, T. R., and E. C. Gotschlich.** 1982. The capsule of *Cryptococcus neoformans* passively inhibits phagocytosis of the yeast by macrophages. J. Immunol. **129:**1675–1680.
148. **Kozel, T. R., and G. S. T. Pfrommer.** 1986. Activation of the complement system by *Cryptococcus neoformans* leads to binding of iC3b to the yeast. Infect. Immun. **52:**1–5.
149. **Kozel, T. R., G. S. T. Pfrommer, A. S. Guerlain, B. A. Highison, and G. J. Highison.** 1988. Role of the capsule in phagocytosis of *Cryptococcus neoformans*. Rev. Infect. Dis. **10:**S436–S439.
150. **Kozel, T. R., M. A. Wilson, T. P. Farrell, and S. M. Levitz.** 1989. Activation of C3 and binding to *Aspergillus fumigatus* conidia and hyphae. Infect. Immun. **57:**3412–3417.
151. **Kozel, T. R., M. A. Wilson, and J. W. Murphy.** 1991. Early events in initiation of alternative complement pathway activation by the capsule of *Cryptococcus neoformans*. Infect. Immun. **59:**3101–3110.
152. **Kruse, D., and G. T. Cole.** 1992. A seroreactive 120-kilodalton β-1,3-glucanase of *Coccidioides immitis* which may participate in spherule morphogenesis. Infect. Immun. **60:**4350–4363.
153. **Kwon-Chung, K. J.** 1979. Comparison of isolates of *Sporothrix schenckii* obtained from fixed cutaneous lesions with isolates from other types of lesioins. J. Infect. Dis. **139:**424–431.
154. **Kwon-Chung, K. J.** 1994. Phylogenetic spectrum of fungi that are pathogenic to humans. Clin. Infect. Dis. **19:**S1–S7.
155. **Kwon-Chung, K. J., and J. E. Bennett.** 1978. Distribution of A and a mating types of *Cryptococcus neoformans* among natural and clinical isolates. Am. J. Epidemiol. **108:**337–340.
156. **Kwon-Chung, K. J., and J. E. Bennett.** 1992. Medical mycology, p. 707–729. Lea & Febiger, Philadelphia.
157. **Kwon-Chung, K. J., J. C. Edman, and B. L. Wickes.** 1992. Genetic association of mating types and virulence in *Cryptococcus neoformans*. Infect. Immun. **60:**602–605.
158. **Kwon-Chung, K. J., W. B. Hill, and J. E. Bennett.** 1981. New, special stain for histopathological diagnosis of cryptococcosis. J. Clin. Microbiol. **13:**383–387.
159. **Kwon-Chung, K. J., I. Polacheck, and T. J. Popkin.** 1982. Melanin-lacking mutants of *Cryptococcus neoformans* and their virulence for mice. J. Bacteriol. **150:**1414–1421.
160. **Kwon-Chung, K. J., and J. C. Rhodes.** 1986. Encapsulation and melanin formation as indicators of virulence in *Cryptococcus neoformans*. Infect. Immun. **51:**218–223.
161. **Kwon-Chung, K. J., W. K. Tom, and J. L. Costa.** 1983. Utilization of indole compounds by *Cryptococcus neoformans* to produce a melanin-like pigment. J. Clin. Microbiol. **18:**1419–1421.
162. **Kwon-Chung, K. J., B. L. Wickes, L. Stockman, G. D. Roberts, D. Ellis, and D. H. Howard.** 1992. Virulence, serotype, and molecular characteristics of environmental strains of *Cryptococcus neoformans* var. *gattii*. Infect. Immun. **60:**1869–1874.
163. **Lamy, B., M. Moutaouakil, J. P. Latge, and J. Davies.** 1991. Secretion of a potential virulence factor, a fungal ribonucleotoxin, during human aspergillosis infections. Mol. Microbiol. **5:**1811–1815.
163a. **Lancet.** 1989. Blastomycosis—one disease or two? Lancet. **i:**25–26. (Editorial.)
164. **Lane, T. E., G. C. Otero, B. A. Wu-Hsieh, and D. H. Howard.** 1994. Expression of inducible nitric oxide synthase by stimulated macrophages correlates with their antihistoplasma activity. Infect. Immun. **62:**1478–1479.
165. **Lane, T. E., B. A. Wu-Hsieh, and D. H. Howard.** 1991. Iron limitation and the gamma interferon-mediated antihistoplasma state of murine macrophages. Infect. Immun. **59:**2274–2278.
166. **Lane, T. E., B. A. Wu-Hsieh, and D. H. Howard.** 1993. Gamma interferon cooperates with lipopolysaccharide to activate mouse splenic macrophages to an antihistoplasma state. Infect. Immun. **61:**1468–1473.
167. **Larcher, G., J. P. Bouchara, V. Annaix, F. Symoens, D. Chabasse, and G. Tronchin.** 1992. Purification and characterization of a fibrinogenolytic serine proteinase from *Aspergillus fumigatus* culture filtrate. FEBS Lett. **308:**65–69.
168. **Latge, J. P., M. Moutaouakil, J. P. Debeaupuis, J. P. Bouchara, K. Haynes, and M. C. Prevost.** 1991. The 18-kilodalton antigen secreted by *Aspergillus fumigatus*. Infect. Immun. **59:**2586–2594.
169. **Lei, P. C., T. Yoshiike, and H. Ogawa.** 1993. Effects of proteinase inhibitors on the cutaneous lesion of *Sporothrix schenckii* inoculated hairless mice. Mycopathologia **123:**81–5.
170. **Levitz, S. M.** 1989. Aspergillosis. Infect. Dis. Clin. North Am. **3:**1–18.
171. **Levitz, S. M.** 1991. The ecology of *Cryptococcus neoformans* and the epidemiology of cryptococcosis. Rev. Infect. Dis. **13:**1163–1169.
172. **Levitz, S. M.** 1994. Macrophage *Cryptococcus* interactions, p. 533–543. *In* B. S. Zwilling and T. K. Eisenstein (ed.), Macrophage pathogen interactions. Marcel Dekker, Inc., New York.
173. **Levitz, S. M., and R. D. Diamond.** 1985. Mechanisms of resistance of *Aspergillus fumigatus* conidia to killing by neutrophils in vitro. J. Infect. Dis. **152:**33–42.
174. **Levitz, S. M., and R. D. Diamond.** 1985. A rapid colorimetric assay of fungal viability with the tetrazolium salt MTT. J. Infect. Dis. **152:**938–945.
175. **Levitz, S. M., and D. J. DiBenedetto.** 1988. Differential stimulation of murine resident peritoneal cells by selectively opsonized encapsulated and acapsular *Cryptococcus neoformans*. Infect. Immun. **56:**2544–2551.
176. **Levitz, S. M., and D. J. DiBenedetto.** 1989. Paradoxical role of capsule in murine bronchoalveolar macrophage-mediated killing of *Cryptococcus neoformans*. J. Immunol. **142:**659–665.
177. **Levitz, S. M., and T. P. Farrell.** 1990. Growth inhibition of *Cryptococcus neoformans* by cultured human monocytes: role of the capsule, opsonins, the culture surface, and cytokines. Infect. Immun. **58:**1201–1209.
178. **Levitz, S. M., and T. P. Farrell.** 1990. Human neutrophil degranulation stimulated by *Aspergillus fumigatus*. J. Leukocyte Biol. **47:**170–175.
179. **Levitz, S. M., T. P. Farrell, and R. T. Maziarz.** 1991. Killing of *Cryptococcus neoformans* by human peripheral blood mononuclear cells stimulated in culture. J. Infect. Dis. **163:**1108–1113.
180. **Levitz, S. M., M. E. Selsted, T. Ganz, R. I. Lehrer, and R. D. Diamond.** 1986. In vitro killing of spores and hyphae of *Aspergillus fumigatus* and *Rhizopus oryzae* by rabbit neutrophil cationic peptides and bronchoalveolar macrophages. J. Infect. Dis. **154:**483–489.
181. **Levitz, S. M., and A. Tabuni.** 1991. Binding of *Cryptococcus neoformans* by human cultured macrophages. Requirements for multiple complement receptors and actin. J. Clin. Invest. **87:**528–535.
182. **Levitz, S. M., A. Tabuni, H. Kornfeld, C. C. Reardon, and D. T. Golenbock.** 1994. Production of tumor necrosis factor alpha in human leukocytes stimulated by *Cryptococcus neoformans*. Infect. Immun. **62:**1975–1981.
183. **Levitz, S. M., A. Tabuni, R. Wagner, H. Kornfeld, and E. H. Smail.** 1992. Binding of unopsonized *Cryptococcus neoformans* by human bronchalveolar macrophages: inhibition by a large-molecular-size serum component. J. Infect. Dis. **166:**866–873.
184. **Littman, M. L., and E. Tsubura.** 1959. Effect of degree of encapsulation upon virulence of *Cryptococcus neoformans*. Proc. Soc. Exp. Biol. Med. **101:**773–777.
185. **Lodge, J. K., E. Jackson-Machelski, D. L. Toffaletti, J. R. Perfect, and J. I. Gordon.** 1994. Targeted gene replacement demonstrates that myristoyl-CoA:protein N-myristoyltransferase is essential for viability of *Cryptococcus neoformans*. Proc. Natl. Acad. Sci. USA **91:**12008–12012.
186. **Loose, D. S., and D. Feldman.** 1982. Characterization of a unique corticosterone-binding protein in *Candida albicans*. J. Biol. Chem. **257:**4925–4930.
187. **Loose, D. S., E. P. Stover, A. Restrepo, D. A. Stevens, and D. Feldman.** 1983. Estradiol binds to a receptor-like cytosol binding protein and initiates a biological response in *Paracoccidioides brasiliensis*. Proc. Natl. Acad. Sci. USA **80:**7659–7663.
188. **Macher, A. B., J. E. Bennett, J. E. Gadek, and M. M. Frank.** 1978. Complement depletion in cryptococcal sepsis. J. Immunol. **120:**1686–1690.
189. **Mackinnon, J. E., and I. A. Conti-Díaz.** 1962. The effect of temperature on sporotrichosis. Sabouraudia **2:**56–59.
190. **Maresca, B., J. Bennett, W. Fonzi, C. A. Hitchcock, J. K. Lodge, and P. R. Williamson.** 1994. Molecular approaches to identify novel targets for future development of antifungal agents. J. Med. Vet. Mycol. **32:**287–298.
191. **Maresca, B., L. Carratù, and G. S. Kobayashi.** 1994. Morphological transition in the human fungal pathogen *Histoplasma capsulatum*. Trends Microbiol. **2:**110–114.
192. **Maresca, B., and G. Kobayashi.** 1993. Changes in membrane fluidity modulate heat shock gene expression and produced attenuated strains in the dimorphic fungus *Histoplasma capsulatum*. Arch. Med. Res. **24:**247–249.
193. **Markaryan, A., I. Morozova, H. Yu, and P. E. Kolattukudy.** 1994. Purification and characterization of an elastinolytic metalloprotease from *Aspergillus fumigatus* and immunoelectron microscopic evidence of secretion of this enzyme by the fungus invading the murine lung. Infect. Immun. **62:**2149–2157.
194. **McEwen, J. G., V. Bedoya, M. M. Patiño, M. E. Salazar, and A. Restrepo M.** 1987. Experimental murine paracoccidioidomycosis induced by the inhala-

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tion of conidia. J. Med. Vet. Mycol. **25:**165–175.
195. **Miller, M. F., and T. G. Mitchell.** 1991. Killing of *Cryptococcus neoformans* strains by human neutrophils and monocytes. Infect. Immun. **59:**24–28.
196. **Miller, R. F., and D. M. Mitchell.** 1995. AIDS and the lung: update 1995. 1. *Pneumocystis carinii* pneumonia. Thorax **50:**191–200.
197. **Mitchell, T. G., and J. R. Perfect.** 1995. Cryptococcosis in the era of AIDS—100 years after the discovery of *Cryptococcus neoformans*. Clin. Microbiol. Rev. **8:**515–548.
198. **Mohr, J. A., H. Long, B. A. McKown, and H. G. Muchmore.** 1972. In vitro susceptibility of *Cryptococcus neoformans* to steroids. Sabouraudia **10:**171–172.
199. **Mondon, P., J. Thélu, B. Lebeau, P. Ambroise-Thomas, and R. Grillot.** 1995. Virulence of *Aspergillus fumigatus* strains investigated by random amplified polymorphic DNA analysis. J. Med. Microbiol. **42:**299–303.
200. **Monod, M., S. Paris, J. Sarfati, K. Jaton-Ogay, P. Ave, and J. P. Latge.** 1993. Virulence of alkaline protease-deficient mutants of *Aspergillus fumigatus*. FEMS Microbiol. Lett. **80:**39–46.
201. **Morrison, C. J., and D. A. Stevens.** 1991. Mechanisms of fungal pathogenicity: correlation of virulence in vivo, susceptibility to killing by polymorphonuclear neutrophils in vitro, and neutrophil superoxide anion induction among *Blastomyces dermatitidis* isolates. Infect. Immun. **59:**2744–2749.
202. **Moser, M., G. Menz, K. Blaser, and R. Crameri.** 1994. Recombinant expression and antigenic properties of a 32-kilodalton extracellular alkaline protease, representing a possible virulence factor from *Aspergillus fumigatus*. Infect. Immun. **62:**936–942.
203. **Moutaouakil, M., M. Monod, M. C. Prevost, J. P. Bouchara, S. Paris, and J. P. Latge.** 1993. Identification of the 33-kDa alkaline protease of *Aspergillus fumigatus* in vitro and in vivo. J. Med. Microbiol. **39:**393–399.
204. **Mukherjee, J., A. Casadevall, and M. D. Scharff.** 1993. Molecular characterization of the humoral responses to *Cryptococcus neoformans* infection and glucuronoxylomannan-tetanus toxoid conjugate immunization. J. Exp. Med. **177:**1105–1116.
205. **Mukherjee, J., G. Nussbaum, M. D. Scharff, and A. Casadevall.** 1995. Protective and nonprotective monoclonal antibodies to *Cryptococcus neoformans* originating from one B cell. J. Exp. Med. **181:**405–409.
206. **Mullbacher, A., and R. D. Eichner.** 1984. Immunosuppression in vitro by a metabolite of a human pathogenic fungus. Proc. Natl. Acad. Sci. USA **81:** 3835–3837.
207. **Mullbacher, A., P. Waring, and R. D. Eichner.** 1985. Identification of an agent in cultures of *Aspergillus fumigatus* displaying anti-phagocytic and immunomodulating activity in vitro. J. Gen. Microbiol. **131:**1251–1258.
208. **Murphy, J. W.** 1988. Influence of cryptococcal antigens on cell-mediated immunity. Rev. Infect. Dis. **10:**S432–S435.
209. **Murphy, J. W., and G. C. Cozad.** 1972. Immunological unresponsiveness induced by cryptococcal capsular polysaccharide assayed by the hemolytic plaque technique. Infect. Immun. **5:**896–901.
210. **Murphy, J. W., H. Friedman, and M. Bendinelli (ed.).** 1993. Fungal infections and immune responses. Plenum Press, New York.
211. **Murphy, J. W., and J. W. Moorhead.** 1982. Regulation of cell-mediated immunity in cryptococcosis. I. Induction of specific afferent T suppressor cells by cryptococcal antigen. J. Immunol. **128:**276–283.
212. **Nakamura, L. T., B. A. Wu-Hsieh, and D. H. Howard.** 1994. Recombinant murine gamma interferon stimulates macrophages of the RAW cell line to inhibit intracellular growth of *Histoplasma capsulatum*. Infect. Immun. **62:** 680–684.
213. **Neilson, J. B., R. A. Fromtling, and G. S. Bulmer.** 1977. *Cryptococcus neoformans*: size range of infectious particles from aerosolized soil. Infect. Immun. **17:**634–638.
214. **Newman, S. L., and W. E. Bullock.** 1994. Interaction of *Histoplasma capsulatum* yeasts and conidia with human and animal macrophages. Immunol. Ser. **60:**517–532.
215. **Newman, S. L., S. Chaturvedi, and B. S. Klein.** 1995. The WI-1 Ag of *Blastomyces dermatitidis* yeasts mediates binding to human macrophage CD11b/CD18 (CR3) and CD14. J. Immunol. **154:**753–761.
216. **Newman, S. L., L. Gootee, G. Brunner, and G. S. Deepe, Jr.** 1994. Chloroquine induces human macrophage killing of Histoplasma capsulatum by limiting the availability of intracellular iron and is therapeutic in a murine model of histoplasmosis. J. Clin. Invest. **93:**1422–1429.
217. **Newman, S. L., L. Gootee, R. Morris, and W. E. Bullock.** 1992. Digestion of Histoplasma capsulatum yeasts by human macrophages. J. Immunol. **149:** 574–580.
218. **Ng, T. T., G. D. Robson, and D. W. Denning.** 1994. Hydrocortisone-enhanced growth of *Aspergillus* spp.: implications for pathogenesis. Microbiology **140:**2475–2479.
219. **Niehaus, W. G., and T. Flynn.** 1994. Regulation of mannitol biosynthesis and degradation by *Cryptococcus neoformans*. J. Bacteriol. **176:**651–655.
220. **Oda, L. M., C. F. Kubelka, C. S. Alviano, and L. R. Tranvassos.** 1983. Ingestion of yeast forms of *Sporothrix schenckii* by mouse peritoneal macrophages. Infect. Immun. **39:**497–504.
221. **Orendi, J. M., H. S. L. M. Nottet, M. R. Visser, A. F. M. Verheul, H. Snippe, and J. Verhoef.** 1994. Enhancement of HIV-1 replication in peripheral blood mononuclear cells by *Cryptococcus neoformans* is monocyte-dependent but tumour necrosis factor-independent. AIDS **8:**423–429.
222. **Paris, S., M. Monod, M. Diaquin, B. Lamy, L. K. Arruda, P. J. Punt, and J. P. Latge.** 1993. A transformant of *Aspergillus fumigatus* deficient in the antigenic cytotoxin ASPFI. FEMS Microbiol. Lett. **111:**31–36.
223. **Parta, M., Y. Chang, S. Rulong, P. Pinto-DaSilva, and K. J. Kwon-Chung.** 1994. HYP1, a hydrophobin gene from *Aspergillus fumigatus*, complements the rodletless phenotype in *Aspergillus nidulans*. Infect. Immun. **62:**4389–4395.
224. **Pendrak, M. L., and S. A. Klotz.** 1995. Adherence of *Candida albicans* to host cells. FEMS Microbiol. Lett. **129:**103–113.
225. **Perfect, J. R.** 1989. Cryptococcosis. Infect. Dis. Clin. North Am. **3:**77–102.
226. **Perfect, J. R., D. L. Toffaletti, and T. H. Rude.** 1993. The gene encoding phosphoribosylaminoimidazole carboxylase (ADE2) is essential for growth of *Cryptococcus neoformans* in cerebrospinal fluid. Infect. Immun. **61:**4446–4451.
227. **Pettoello-Mantovani, M., A. Casadevall, T. R. Kollman, A. Rubinstein, and H. Goldstein.** 1992. Enhancement of HIV-1 infection by the capsular polysaccharide of *Cryptococcus neoformans*. Lancet **339:**21–23.
228. **Pettoello-Mantovani, M., A. Casadevall, P. Smarnworawong, and H. Goldstein.** 1994. Enhancement of HIV type 1 infectivity *in vitro* by capsular polysaccharide of *Cryptococcus neoformans* and *Haemophilus influenzae*. AIDS Res. Hum. Retroviruses **10:**1079–1087.
229. **Polacheck, I.** 1991. The discovery of melanin production in *Cryptococcus neoformans* and its impact on diagnosis and the study of virulence. Int. J. Med. Microbiol. **276:**120–123.
230. **Polacheck, I., V. J. Hearing, and K. J. Kwon-Chung.** 1982. Biochemical studies of phenoloxidase and utilization of catecholamines in *Cryptococcus neoformans*. J. Bacteriol. **150:**1212–1220.
231. **Polacheck, I., Y. Platt, and J. Aronovitch.** 1990. Catecholamines and virulence of *Cryptococcus neoformans*. Infect. Immun. **58:**2919–2922.
232. **Polak, A.** 1990. Melanin as a virulence factor in pathogenic fungi. Mycoses **33:**215–224.
233. **Powell, B. L., and D. A. Drutz.** 1984. Identification of a high-affinity binder for estradiol and a low-affinity binder for testosterone in *Coccidioides immitis*. Infect. Immun. **45:**784–786.
234. **Powell, B. L., D. J. Drutz, M. Huppert, and S. H. Sun.** 1983. Relationship of progesterone- and estradiol-binding proteins in *Coccidioides immitis* to coccidioidal dissemination in pregnancy. Infect. Immun. **40:**478–485.
235. **Puccia, R., S. Schenkman, P. A. Gorin, and L. R. Travassos.** 1986. Exocellular components of *Paracoccidioides brasiliensis*: identification of a specific antigen. Infect. Immun. **53:**199–206.
236. **Puccia, R., and L. R. Travassos.** 1991. The 43-kDa glycoprotein from the human pathogen Paracoccidioides brasiliensis and its deglycosylated form: excretion and susceptibility to proteolysis. Arch. Biochem. Biophys. **289:** 298–302.
237. **Ramesh, M. V., T. Sirakova, and P. E. Kolattukudy.** 1994. Isolation, characterization, and cloning of cDNA and the gene for an elastinolytic serine proteinase from *Aspergillus flavus*. Infect. Immun. **62:**79–85.
238. **Reichard, U., H. Eiffert, and R. Rüchel.** 1994. Purification and characterization of an extracellular aspartic proteinase from *Aspergillus fumigatus*. J. Med. Vet. Mycol. **32:**427–436.
239. **Remaley, A. T., D. B. Kuhns, R. E. Basford, R. H. Glen, and S. S. Kaplan.** 1984. Leishmanaial phosphatase blocks neutrophil O^{2-} production. J. Biol. Chem. **259:**11173–11175.
240. **Resnick, S., D. Pappagianis, and J. H. McKerrow.** 1987. Proteinase production by the parasitic cycle of the pathogenic fungus *Coccidioides immitis*. Infect. Immun. **55:**2807–2815.
241. **Restrepo, A., M. E. Salazar, L. E. Cano, E. P. Stover, D. Feldman, and D. A. Stevens.** 1984. Estrogens inhibit mycelium-to-yeast transformation in the fungus *Paracoccidioidomycosis brasiliensis*: implications for resistance of females to paracoccidioidomycosis. Infect. Immun. **46:**346–353.
242. **Restrepo-Moreno, A.** 1993. Paracoccidioidomycosis, p. 251–276. *In* J. W. Murphy, H. Friedman, and M. Bendinelli (ed.), Fungal infections and immune responses. Plenum Press, New York.
243. **Restrepo-Moreno, A., and D. L. Greer.** 1983. Paracoccidioidomycosis, p. 43–64. *In* A. F. DiSalvo (ed.), Occupational mycoses. Lea & Febiger, Philadelphia.
244. **Rhodes, J. C., T. W. Amlung, and M. S. Miller.** 1990. Isolation and characterization of an elastinolytic proteinase from *Aspergillus flavus*. Infect. Immun. **58:**2529–2534.
245. **Rhodes, J. C., R. B. Bode, and C. M. McCuan-Kirsch.** 1988. Elastase production in clinical isolates of *Aspergillus*. Diagn. Microbiol. Infect. Dis. **10:**165–170.
246. **Rhodes, J. C., I. Polacheck, and K. J. Kwon-Chung.** 1982. Phenoloxidase activity and virulence in isogenic strains of *Cryptococcus neoformans*. Infect. Immun. **36:**1175–1184.
247. **Richardson, M. D., L. J. White, I. C. McKay, and G. S. Shankland.** 1993. Differential binding of acapsulate and encapsulated strains of *Cryptococcus neoformans* to human neutrophils. J. Med. Vet. Mycol. **31:**189–199.
248. **Rippon, J. W.** 1982. Sporotrichosis, p. 277–302. *In* J. W. Rippon (ed.), Medical mycology. The W. B. Saunders Co., Philadelphia.
249. **Robens, J. F., and J. L. Richard.** 1992. Aflatoxins in animal and human

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health. Rev. Environ. Contam. Toxicol. **127:**69–94.
250. **Robertson, M. D., A. Seaton, L. J. Milne, and J. A. Raeburn.** 1987. Resistance of spores of *Aspergillus fumigatus* to ingestion by phagocytic cells. Thorax **42:**466–472.
251. **Robertson, M. D., A. Seaton, L. J. Milne, and J. A. Raeburn.** 1987. Suppression of host defences by *Aspergillus fumigatus*. Thorax **42:**19–25.
252. **Roos, D.** 1994. The genetic basis of chronic granulomatous disease. Immunol. Rev. **138:**121–157.
253. **Ryter, A., and H. Fromentin.** 1985. Ultrastructural study of the interaction of the fungi *Sporothrix schenckii* and *Ceratocystis stenoceras* with bone-marrow-derived murine macrophages. Microbiologie **136B:**9–27.
254. **Salazar, M. E., A. Restrepo, and D. A. Stevens.** 1988. Inhibition by estrogens of conidium-to-yeast conversion in the fungus *Paracoccidioides brasiliensis*. Infect. Immun. **56:**711–713.
255. **San-Blas, F., and G. San-Blas.** 1992. Mutants of *Paracoccidioides brasiliensis* strain IVIC Pb9 affected in dimorphism. J. Med. Vet. Mycol. **30:**51–60.
256. **San-Blas, F., G. San-Blas, J. Hallak, and E. Merino.** 1983. Ultrastructure and cell wall chemistry of a thermosensitive mutant of *Paracoccidioides brasiliensis*. Curr. Microbiol. **8:**85–88.
257. **San-Blas, G.** 1993. Paracoccidioidomycosis and its etiologic agent Paracoccidioides brasiliensis. J. Med. Vet. Mycol. **31:**99–113.
258. **San-Blas, G., A. Restrepo, K. Clemons, D. A. Stevens, F. San-Blas, R. Puccia, L. R. Travassos, J. I. Figueroa, A. J. Hamilton, M. A. Bartholomew, T. Harada, L. Fenelon, and R. J. Hay.** 1992. Paracoccidioidomycosis. J. Med. Vet. Mycol. **30:**59–71.
259. **San-Blas, G., and F. San-Blas.** 1977. *Paracoccidioides brasiliensis*: cell wall structure and virulence. Mycopathologia **62:**77–86.
260. **Sandhu, D. K., R. S. Sandhu, Z. U. Khan, and V. N. Damodaran.** 1976. Conditional virulence of a *p*-aminobenzoic acid-requiring mutant of *Aspergillus fumigatus*. Infect. Immun. **13:**527–532.
261. **Sano, A., M. Miyaji, and K. Nishimura.** 1991. Studies on the relationship between paracoccidioidomycosis in ddY mice and their estrous cycle. Mycopathologia **115:**73–81.
262. **Schaffner, A., H. Douglas, and A. Braude.** 1982. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to *Aspergillus*. Observations on these two lines of defense in vivo and in vitro with human and mouse phagocytes. J. Clin. Invest. **69:** 617–631.
263. **Silva, C. L., L. M. Alves, and F. Figueiredo.** 1994. Involvement of cell wall glucans in the genesis and persistence of the inflammatory reaction caused by the fungus *Paracoccidioides brasiliensis*. Microbiology—UK. **140:**1189–1194.
264. **Silva, C. L., and R. A. Fazioli.** 1985. A *Paracoccidioides brasiliensis* polysaccharide having granuloma-inducing toxic and macrophage-stimulating activity. J. Gen. Microbiol. **131:**1497–1501.
265. **Silva, C. L., and F. Figueiredo.** 1991. Tumor necrosis factor in paracoccidioidomycosis patients. J. Infect. Dis. **164:**1033–1034.
266. **Sirakova, T. D., A. Markaryan, and P. E. Kolattukudy.** 1994. Molecular cloning and sequencing of the cDNA and gene for a novel elastinolytic metalloproteinase from *Aspergillus fumigatus* and its expression in *Escherichia coli*. Infect. Immun. **62:**4208–4218.
267. **Small, J. M., and T. G. Mitchell.** 1989. Strain variation in antiphagocytic activity of capsular polysaccharides from *Cryptococcus neoformans* serotype A. Infect. Immun. **57:**3751–3756.
268. **Smith, J. M., J. E. Davies, and D. W. Holden.** 1993. Construction and pathogenicity of *Aspergillus fumigatus* mutants that do not produce the ribotoxin restrictocin. Mol. Microbiol. **9:**1071–1077.
269. **Smith, J. M., C. M. Tang, S. Van Noorden, and D. W. Holden.** 1994. Virulence of *Aspergillus fumigatus* double mutants lacking restriction and an alkaline protease in a low-dose model in invasive pulmonary aspergillosis. Infect. Immun. **62:**5247–5254.
270. **Speed, B., and D. Dunt.** 1995. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. Clin. Infect. Dis. **21:**28–34.
271. **Staib, F.** 1962. Cryptococcus neoformans und Guizotia abyssinica. Zentralbl. Hyg. Umweltmed. **148:**466–475.
272. **Stevens, D. A.** 1989. The interface of mycology and endocrinology. J. Med. Vet. Mycol. **27:**133–140.
273. **Stover, E. P., G. Schar, K. V. Clemons, D. A. Stevens, and D. Feldman.** 1986. Estradiol-binding proteins from mycelial and yeast-form cultures of *Paracoccidioides brasiliensis*. Infect. Immun. **51:**199–203.
274. **Sturtevant, J., and J. P. Latge.** 1992. Participation of complement in the phagocytosis of the conidia of *Aspergillus fumigatus* by human polymorphonuclear cells. J. Infect. Dis. **166:**580–586.
275. **Sturtevant, J. E., and J. P. Latge.** 1992. Interactions between conidia of *Aspergillus fumigatus* and human complement component C3. Infect. Immun. **60:**1913–1918.
276. **Sugar, A. M.** 1992. Mucormycosis. Clin. Infect. Dis. **14**(Suppl. 1)**:**S126–S129.
277. **Sundstrom, J. B., and R. Cherniak.** 1993. T-cell-dependent and T-cell-independent mechanisms of tolerance to glucuronoxylomannan of *Cryptococcus neoformans* serotype A. Infect. Immun. **61:**1340–1345.
278. **Sutton, P., N. R. Newcombe, P. Waring, and A. Mullbacher.** 1994. In vivo immunosuppressive activity of gliotoxin, a metabolite produced by human pathogenic fungi. Infect. Immun. **62:**1192–1198.
279. **Taba, M. R. M., J. F. D. Silveira, L. R. Travassos, and S. Schenkman.** 1989. Expression in *Escherichia coli* of a gene coding for epitopes of a diagnostic antigen of *Paracoccidioides brasiliensis*. Exp. Mycol. **13:**223–230.
280. **Tang, C. M., J. Cohen, and D. W. Holden.** 1992. An *Aspergillus fumigatus* alkaline protease mutant constructed by gene disruption is deficient in extracellular elastase activity. Mol. Microbiol. **6:**1663–1671.
281. **Tang, C. M., J. Cohen, T. Krausz, S. Van Noorden, and D. W. Holden.** 1993. The alkaline protease of *Aspergillus fumigatus* is not a virulence determinant in two murine models of invasive pulmonary aspergillosis. Infect. Immun. **61:**1650–1656.
282. **Tang, C. M., J. M. Smith, H. N. Arst, Jr., and D. W. Holden.** 1994. Virulence studies of *Aspergillus nidulans* mutants requiring lysine or *p*-aminobenzoic acid in invasive pulmonary aspergillosis. Infect. Immun. **62:**5255–5260.
283. **Taylor, M. W., and G. S. Feng.** 1991. Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism. FASEB J. **5:**2516–2522.
284. **Thau, N., M. Monod, B. Crestani, C. Rolland, G. Tronchin, J. P. Latge, and S. Paris.** 1994. Rodletless mutants of *Aspergillus fumigatus*. Infect. Immun. **62:**4380–4388.
285. **Torres-Guererro, H., and J. C. Edman.** 1994. Melanin-deficient mutants of *Cryptococcus neoformans*. J. Med. Vet. Mycol. **32:**303–313.
286. **Tronchin, G., J. P. Bouchara, V. Annaix, R. Robert, and J. M. Senet.** 1991. Fungal cell adhesion molecules in *Candida albicans*. Eur. J. Epidemiol. **7:**23–33.
287. **Tronchin, G., J. P. Bouchara, G. Larcher, J. C. Lissitzky, and D. Chabasse.** 1993. Interaction between *Aspergillus fumigatus* and basement membrane laminin: binding and substrate degradation. Biol. Cell. **77:**201–208.
288. **Truelsen, K., T. Young, and T. R. Kozel.** 1992. In vivo complement activation and binding of C3 to encapsulated *Cryptococcus neoformans*. Infect. Immun. **60:**3937–3939.
289. **Tsuboi, R., T. Sanada, and H. Ogawa.** 1988. Influence of culture medium pH and proteinase inhibitors on extracellular proteinase activity and cell growth of *Sporothrix schenckii*. J. Clin. Microbiol. **26:**1431–1433.
290. **Tsuboi, R., T. Sanada, K. Takamori, and H. Ogawa.** 1987. Isolation and properties of extracellular proteinases from *Sporothrix schenckii*. J. Bacteriol. **169:**4104–4109.
291. **Van Cutsem, J., and J. R. Boelaert.** 1989. Effects of deferoxamine, feroxamine and iron on experimental mucormycosis (zygomycosis). Kidney Int. **36:**1061–1068.
292. **Vartivarian, S. E., E. J. Anaissie, R. E. Cowart, H. A. Sprigg, M. J. Tingler, and E. S. Jacobson.** 1993. Regulation of cryptococcal capsular polysaccharide by iron. J. Infect. Dis. **167:**186–190.
293. **Vartivarian, S. E., R. E. Cowart, E. J. Anaissie, T. Tashiro, and H. A. Sprigg.** 1995. Iron acquisition by *Cryptococcus neoformans*. J. Med. Vet. Mycol. **33:**151–156.
294. **Vaz, C. A. C., D. W. R. MacKenzie, V. Hearn, Z. P. Camargo, L. M. Singer-Vermes, E. Burger, and V. L. G. Calich.** 1994. Gelatinase activity of exoantigens from virulent and non-virulent *Paracoccidioides brasiliensis*. J. Med. Vet. Mycol. **32:**65–69.
295. **Vecchiarelli, A., D. Pietrella, M. Dottorini, C. Monari, C. Retini, T. Todisco, and F. Bistoni.** 1994. Encapsulation of *Cryptococcus neoformans* regulates fungicidal activity and the antigen presentation process in human alveolar macrophages. Clin. Exp. Immunol. **98:**217–223.
296. **Vecchiarelli, A., C. Retini, D. Pietrella, C. Monari, C. Tascini, T. Beccari, and T. R. Kozel.** 1995. Downregulation by cryptococcal polysaccharide of tumor necrosis factor alpha and interleukin-1B secretion from human monocytes. Infect. Immun. **63:**2919–2923.
297. **Verdonck, A. K., J. R. Boelaert, B. Z. Gordts, and H. W. Van Landuyt.** 1993. Effect of ferrioxamine on the growth of *Rhizopus*. Mycoses **36:**9–12.
298. **Vicentini, A. P., J. L. Gesztesi, M. F. Franco, W. de Souza, J. Z. de Moraes, L. R. Travassos, and J. D. Lopes.** 1994. Binding of *Paracoccidioides brasiliensis* to laminin through surface glycoprotein gp43 leads to enhancement of fungal pathogenesis. Infect. Immun. **62:**1465–1469.
299. **Waldorf, A. R., and R. D. Diamond.** 1984. Cerebral mucormycosis in diabetic mice after intrasinus challenge. Infect. Immun. **44:**194–195.
300. **Waldorf, A. R., and R. D. Diamond.** 1985. Neutrophil chemotactic responses induced by fresh and swollen *Rhizopus oryzae* spores and *Aspergillus fumigatus* conidia. Infect. Immun. **48:**458–463.
301. **Waldorf, A. R., S. M. Levitz, and R. D. Diamond.** 1984. In vivo bronchoalveolar macrophage defense against *Rhizopus oryzae* and *Aspergillus fumigatus*. J. Infect. Dis. **150:**752–760.
302. **Waldorf, A. R., N. Ruderman, and R. D. Diamond.** 1984. Specific susceptibility to mucromycosis in murine diabetes and bronchoalveolar macrophage defense against *Rhizopus*. J. Clin. Invest. **74:**150–160.
303. **Wang, Y., P. Aisen, and A. Casadevall.** 1995. *Cryptococcus neoformans* melanin and virulence: mechanism of action. Infect. Immun. **63:**3131–3136.
304. **Wang, Y., and A. Casadevall.** 1994. Decreased susceptibility of melanized

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Cryptococcus neoformans to UV light. Appl. Environ. Microbiol. **60:**3864–3866.

305. **Wang, Y., and A. Casadevall.** 1994. Susceptibility of melanized and nonmelanized *Cryptococcus neoformans* to nitrogen- and oxygen-derived oxidants. Infect. Immun. **62:**3004–3007.
306. **Waring, P.** 1990. DNA fragmentation induced in macrophages by gliotoxin does not require protein synthesis and is preceded by raised inositol triphosphate levels. J. Biol. Chem. **265:**14476–14480.
307. **Waring, P., R. D. Eichner, A. Mullbacher, and A. Sjaarda.** 1988. Gliotoxin induces apoptosis in macrophages unrelated to its antiphagocytic properties. J. Biol. Chem. **263:**18493–18499.
308. **Washburn, R. G., B. J. Bryant-Varela, N. C. Julian, and J. E. Bennett.** 1991. Differences in *Cryptococcus neoformans* capsular polysaccharide structure influence assembly of alternative complement pathway C3 convertase on fungal surfaces. Mol. Immunol. **28:**465–470.
309. **Washburn, R. G., D. J. DeHart, D. E. Agwu, B. J. Bryant-Varela, and N. C. Julian.** 1990. *Aspergillus fumigatus* complement inhibitor: production, characterization, and purification by hydrophobic interaction and thin-layer chromatography. Infect. Immun. **58:**3508–3515.
310. **Washburn, R. G., C. H. Hammer, and J. E. Bennett.** 1986. Inhibition of complement by culture supernatants of *Aspergillus fumigatus*. J. Infect. Dis. **154:**944–951.
311. **West, J. L., T. K. Vanheyningen, G. S. Deepe, and W. E. Goldman.** 1992. A calcium-binding protein is a major released product of *Histoplasma capsulatum* yeasts, abstr. F-42, p. 505. *In* Abstracts of the 92nd General Meeting of the American Society for Microbiology 1992. American Society for Microbiology, Washington, D.C.
312. **Williamson, P. R.** 1994. Biochemical and molecular characterization of the diphenol oxidase of *Cryptococcus neoformans*: identification as a laccase. J. Bacteriol. **176:**656–664.
313. **Wong, B., J. R. Perfect, S. Beggs, and K. A. Wright.** 1990. Production of the hexitol D-mannitol by *Cryptococcus neoformans* in vitro and in rabbits with experimental meningitis. Infect. Immun. **58:**1664–1670.
314. **Wood, G. E.** 1992. Mycotoxins in foods and feeds in the United States. J. Anim. Sci. **70:**3941–3949.
315. **Woods, J. P., and W. E. Goldman.** 1992. In vivo generation of linear plasmids with addition of telomeric sequences by *Histoplasma capsulatum*. Mol. Microbiol. **6:**3603–3610.
316. **Woods, J. P., and W. E. Goldman.** 1993. Autonomous replication of foreign DNA in *Histoplasma capsulatum*: role of native telomeric sequences. J. Bacteriol. **175:**636–641.
317. **Wright, S. D., R. A. Ramos, A. Hermanowski-Vosatka, P. Rockwell, and P. A. Detmers.** 1991. Activation of the adhesive capacity of CR3 on neutrophils by endotoxin: dependence on lipopolysaccharide binding protein and CD14. J. Exp. Med. **173:**1281–1286.
318. **Wright, S. D., R. A. Ramos, P. S. Tobias, R. J. Ulevitch, and J. C. Mathison.** 1990. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science **249:**1431.
319. **Wu-Hsieh, B., and D. H. Howard.** 1993. Histoplasmosis, p. 213–250. *In* J. W. Murphy, H. Friedman, and M. Bendinelli (ed.), Fungal infections and immune responses. Plenum Press, New York.
320. **Yoshiike, T., P. C. Lei, H. Komatsuzaki, and H. Ogawa.** 1993. Antibody raised against extracellular proteinases of *Sporothrix schenckii* in *S. schenckii* inoculated hairless mice. Mycopathologia **123:**69–73.
321. **Young, B. J., and T. R. Kozel.** 1993. Effects of strain variation, serotype, and structural modification on kinetics for activation and binding of C3 to *Cryptococcus neoformans*. Infect. Immun. **61:**2966–2972.
322. **Yuan, L., and G. T. Cole.** 1989. Characterization of a proteinase inhibitor isolated from the fungal pathogen *Coccidioides immitis*. Biochem. J. **257:**729–736.
323. **Yuan, L., and G. T. Cole.** 1987. Isolation and characterization of an extracellular proteinase of *Coccidioides immitis*. Infect. Immun. **55:**1970–1978.
324. **Yuan, L., G. T. Cole, and S. H. Sun.** 1988. Possible role of a proteinase in endosporulation of *Coccidioides immitis*. Infect. Immun. **56:**1551–1559.
325. **Zacharias, D., A. Ueda, M. Moscardi-Bacchi, M. Franco, and G. San-Blas.** 1986. A comparative histopathological, immunological, and biochemical study of experimental intravenous paracoccidioidomycosis induced in mice by three *Paracoccidioides brasiliensis* isolates. J. Med. Vet. Mycol. **24:**445–454.
326. **Zebedee, S. L., R. K. Koduri, J. Mukherjee, S. Mukherjee, S. Lee, D. F. Sauer, M. D. Scharff, and A. Casadevall.** 1994. Mouse-human immunoglobulin G1 chimeric antibodies with activities against *Cryptococcus neoformans*. Antimicrob. Agents Chemother. **38:**1507–1514.

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