ASPECTS OF FUNGAL PATHOGENESIS IN HUMANS

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Abstract Fungal diseases have become increasingly important in the past few years. Because few fungi are professional pathogens, fungal pathogenic mechanisms tend to be highly complex, arising in large part from adaptations of preexisting characteristics of the organisms’ nonparasitic lifestyles. In the past few years, genetic approaches have elucidated many fungal virulence factors, and increasing knowledge of host reactions has also clarified much about fungal diseases. The literature on fungal pathogenesis has grown correspondingly; this review, therefore, will not attempt to provide comprehensive coverage of fungal disease but focuses on properties of the infecting fungus and interactions with the host. These topics have been chosen to make the review most useful to two kinds of readers: fungal geneticists and molecular biologists who are interested in learning about the biological problems posed by infectious diseases, and physicians who want to know the kinds of basic approaches available to study fungal virulence.

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INTRODUCTION

Virulence is a complex interrelationship between the infecting organism and the host. Pathogenesis involves interaction (and sometimes modification) of factors on both sides. This is particularly true of fungal pathogenesis. There is no single factor that causes or permits these organisms to be agents of diseases that range from superficial through invasive human infection (32, 64). This review focuses on the pathogenesis of systemic fungal infections in humans. However, we pay attention where appropriate to localized infections caused by fungi that are important systematic pathogens. An example is thrush in AIDS patients, a limited manifestation of infection by the major fungal yeast pathogen of humans, Candida albicans. We divide our discussion into two parts, the first concentrating on properties of the infecting fungus, and the second on the interactions with the host.

THE INFECTING AGENT

Fungal pathogens can be divided into two general classes, primary pathogens and opportunistic pathogens. Fungi in the former class usually have an environmental reservoir and infect individuals who have either been exposed to a large dose or who are immunologically naive to the fungus. Opportunistic pathogens take advantage of debilitated or immunocompromised hosts to cause infection. They may have an environmental reservoir (e.g., Cryptococcus neoformans, Aspergillus fumigatus) or exist as commensals in healthy organisms (e.g., Candida species).

The mechanisms of fungal pathogenesis are much less-well understood than are those of bacterial pathogens. In contrast to bacteria, few fungi are professional pathogens. For the most part, they exist either as saprophytes in nonanimal settings or as commensals, coexisting with the host without any negative consequences. Several are capable of infecting healthy hosts and causing severe systemic disease. Model examples of such primary pathogens include Coccidioides immitis and Histoplasma capsulatum, whose characteristic diseases have been known for many years. Opportunistic human fungal pathogens have become increasingly important over the past 20 years, paradoxically because the success of modern medical practice has led to the survival of debilitated and immunosuppressed patients. Such patients are highly susceptible to infections by opportunistic pathogens such as Candida species, C. neoformans, A. fumigatus and other Aspergillus species, and the zygomycetes.
The fact that the condition of the patient is a major factor in a great many fungal infections makes evident the fact that fungal disease often involves a complex interplay between host and pathogen. Indeed, it seems clear that fungal diseases, like all infectious disease, are dynamic processes. With at least two of the opportunistic pathogens, *C. albicans* and *C. neoformans*, there is evidence of change among the population of the invading microorganisms, with a subset of individual organisms being selected during the process of infection and invasion. Thus, in this case at least, the genetic composition of the pathogen population may be different at the end of the infection compared with the beginning. Whether there are corresponding pathogen-specific changes (which would have to be phenotypic rather than genotypic) in the host is unclear, although the standard immunological-response pathways are of course induced by infection and play important roles in both resistance and pathogenesis.

**Primary Pathogens**

We have chosen to consider primary pathogens as those fungi that cause disease in noncompromised patients. This distinction is necessarily a gray one, because *C. neoformans*, a model opportunist, sometimes causes disease in healthy individuals, and the primary pathogens, such as *C. immitis*, are much more virulent in immunocompromised patients. Furthermore, infection by a primary pathogen often leads to subclinical disease. However, the distinction is worth making in the effort to understand general mechanisms of pathogenesis.

**Opportunistic Pathogens**

Opportunistic pathogens incite disease in hosts whose local or systemic immune attributes have been impaired, damaged, or are innately dysfunctional. The pathogenesis of opportunistic infections involves production of virulence factors that allow individual organisms to be commensals during times when humans have normal immune systems. Then, as the immune system wanes, the organism takes its opportunity of being in the right place at the right time to continue growth. This unregulated growth then leads to invasive infection.

**POTENTIAL VIRULENCE FACTORS**

Because of the complex nature of the host-fungus interaction, there are few factors that are absolutely required for fungal virulence. However, some properties are frequently associated with pathogenesis across the fungal kingdom, and some have been found to be important for specific pathogens. We discuss examples of both kinds. We favor evidence based upon differential virulence in isogenic strains (strains that differ at one genetic locus) differing in the property, rather than correlatory studies. Construction of isogenic strains has become possible in most of the pathogenic fungi in the past several years. In addition, it is important
to remember that because pathogenesis is complex, possession of a single putative virulence factor is not likely to render a fungus pathogenic; a complex mix of properties is usually required. The most convincing evidence for the importance of a potential virulence factor is to show simultaneous loss of the factor and loss of virulence, together with the regain of virulence when the property is restored.

**Growth at Elevated Temperatures**

The ability to grow at body temperature, 37°C, and within the fever range, 38°–42°C, of the human host is clearly an important requirement for systemic infection. This property is not a simple genetic one; experiments with *Saccharomyces cerevisiae* variants able to grow at physiological temperatures suggest that it is a multigenic trait (96). *S. cerevisiae* is normally not pathogenic, but McCusker and coworkers have found that strains, which are able to grow at 42°C and form pseudohyphae, are able to infect and persist in mice. This property is highly correlated with the high-temperature growth phenotype.

In *C. neoformans*, the Ca²⁺-dependent protein phosphatase calcineurin is required for growth at 37°C, as shown by the sensitivity of the organism to cyclosporin and FK506 at 37°C and not at 24°C and by the failure of a strain with a disruption in the catalytic subunit of calcineurin to grow at 37°C. Laboratory strains in which the calcineurin gene has been disrupted are avirulent in an animal model of cryptococcal meningitis (110, 111).

**Adherence**

For most fungal infections, the ability of the host to resist the physical clearing of the infectious agent is important. For example, the lungs have effective means of clearing foreign particles, but *C. immitis*, *Aspergillus* species, *H. capsulatum*, and *C. neoformans* all infect via the bronchial route and must avoid clearance. *C. albicans* also must adhere to various host surfaces both as a commensal to avoid being washed out of its various niches and as a pathogen during the onset of hematogenous infections.

A large number of studies have demonstrated the importance of adherence in various pathogens. A 120-kDa cell wall adhesin, WI-1, which contains 34 copies of a 25–amino acid tandem repeat, has been isolated from the surface of all *Blastomyces dermatitidis* examined. This adhesin mediates attachment to human monocyte-derived macrophages mainly through binding complement type 3 receptors. This protein is both released into the growth medium and found on the cell wall; free WI-1 seems to be recaptured by the fungus and binds with the cell wall via covalent and noncovalent interactions (16). The disruption of the gene prevents binding to and infection of macrophages, diminishes adhesion, and attenuates the virulence of the fungus (17, 76). Hence, in blastomycosis a single molecule affects adhesion both at the site of entry and during later stages of infection.
Adhesion in *C. albicans* has been the focus of much investigation, and several gene products have been implicated. Three of the most intensely studied adhesion mechanisms involve the *HWP1* gene product, the *ALS* gene family, and the *INT1* gene product.

The *HWP1* gene was identified by Staab et al as a partial cDNA fragment from a library of fungal-specific transcripts containing tandem repeats of glutamine and proline residues (141). The complete gene was cloned and then shown to mediate binding to buccal epithelial cells by a novel mechanism involving transglutamination (140, 142, 149). The virulence of a strain with both alleles disrupted was greatly attenuated (155).

The *ALS* gene family has at least four genes. The *ALS1* (agglutinin-like sequence) gene of *C. albicans* encodes a protein similar to alpha-agglutinin, a cell-surface adhesion glycoprotein involved in mating of *S. cerevisiae*, except that *ALS1* has a central domain consisting of a tandemly repeated 36-amino acid sequence. Genomic Southern blots from several *C. albicans* isolates indicate that the number of copies of the tandem repeat element in *ALS1* differs across strains and, in some cases, between *ALS1* alleles in the same strain, suggesting a strain-dependent variability in *ALS1* protein size (69). The *ALA1* gene was identified in a screen of *C. albicans* genes able to confer adhesion on *S. cerevisiae*; *ALA1* is also a member of the *ALS* gene family, because it has homology to the *C. albicans ALS1* protein and the *S. cerevisiae* alpha-agglutinin protein (53). When transformed into a nonadherent strain of *S. cerevisiae*, the agglutinin-like cell surface proteins induced a flocculation phenotype (48, 50). The *ALS2* and *ALS4* genes in the growing *ALS* family of *C. albicans* have been isolated by polymerase chain reaction (PCR) screening of fosmids. *ALS4* expression was correlated with the growth phase of the culture; *ALS2* expression was not observed under many different in vitro growth conditions (68). Northern blot analysis demonstrates that *ALS1* expression varies, depending on which *C. albicans* strain is examined, and that *ALS3* is hyphal specific; both are cell surface glycoproteins (67).

The existence of integrin-like proteins in *C. albicans* has been postulated because monoclonal antibodies to the human leukocyte integrins alpha M and alpha X bind to blastospores and germ tubes, recognize a candidal surface protein of approximately 185 kDa, and inhibit candidal adhesion to human epithelium. The gene alpha *INT1* was isolated from a library of *C. albicans* genomic DNA by screening with a cDNA probe from the transmembrane domain of human alpha M (52). Disruption of *INT1* in *C. albicans* suppresses hyphal growth, adhesion to epithelial cells, and virulence in mice. Thus, *INT1* links adhesion, filamentous growth, and pathogenicity in *C. albicans*, and Int1p may be an attractive target for the development of antifungal therapy (51).

A genomic screen, using insertion mutagenesis, of *Candida glabrata* yielded a mutant that was unable to adhere to cultured laryngeal cells. The mutant was not affected in virulence, suggesting that there are multiple adhesins involved in pathogenesis in this yeast (28a).
Penetration and Dissemination Factors

The first step in fungal infection is introduction of the agent to the host. Infections may be limited to portal of entry or they may become systemic, disseminating either via hematogenous or contiguous routes. Movement from the infecting surface into the bloodstream requires tissue damage. This damage can be preexisting or can occur either by mechanical penetration or new tissue necrosis. Hence, the ability of fungi to penetrate host cells is crucial for progression of infection in the setting of intact skin or gut barriers. For Candida, it is the ability of hyphae to grow through host cell walls that is proposed to account for the importance of polymorphism in virulence. A. fumigatus and other true molds are able to penetrate blood vessels and grow along the vessel lumen as they invade tissue (12) and can use the same or different virulence factors in that process to cross layers of tissue, unlike bacteria whose infections conform to tissue planes.

Hyphae respond thigmotropically (movement toward or away from a touch stimulus) and morphologically to cues such as the presence of a surface, pores, grooves, and ridges. Growth on some firm surfaces elicits a helical growth response. Hyphae follow grooves and ridges of inert substrates and penetrate pores of filtration membranes. Thus, thigmotropism may enhance the ability of a hypha to invade epithelia of a host at sites of weakened integrity or to follow vasculature (60).

Fungi may also spread from the site of infection throughout the host by such mechanisms as host phagocytosis. C. albicans invades endothelial cells through being phagocytosed (46). H. capsulatum is phagocytosed by macrophages but does not seem to be killed and multiplies within the phagosome (42), a characteristic shared by the closely related fungus Blastomyces dermatitidis.

Nutritional and Metabolic Factors

In order to flourish in the host, fungi need to be able to carry out biosynthetic reactions while concentrating relatively scarce nutrients like Ca$^{2+}$ and Fe$^{2+}$. Experiments with auxotrophic mutants of C. albicans (auxotrophs require a nutrient that the parent organism, the prototroph, does not require) have shown that the inability to synthesize purines, pyrimidines, or heme de novo significantly diminishes virulence (75). In fact, auxotrophy for almost any amino acid affects C. albicans virulence (B.B. Magee & J. Becker, unpublished data). An uracil auxotroph of Histoplasma capsulatum is virulent in both a cultured macrophage and a mouse pulmonary colonization model (131a). Strains of C. neoformans unable to synthesize adenine are avirulent and will not produce meningitis in the rabbit model (115).

The ability to synthesize fatty acids has also been shown to be essential for C. albicans both in a systemic mouse model and in a rat model of oropharyngeal disease. Deletion of the FAS2 gene led to an avirulent Candida strain that was an auxotroph for several fatty acids. The heterozygote was also diminished in its virulence (171, 172).
Glycolytic enzymes constitute a group of \textit{C. albicans} proteins that are immunogenic during oral and esophageal infections (151). Changes in glycolytic gene expression accompany the dimorphic transition in \textit{C. albicans} and reflect the underlying physiological status of the cells during morphogenesis (150). Also, there is a broad spectrum of variability in the expression of genes controlling the utilization of alternative carbon and nitrogen sources (125).

Urease production has been shown to be a virulence factor in \textit{C. neoformans} in mouse intravenous and inhalation models but not in rabbit meningitis models (30a). The authors suggest that this enzyme may not be needed for maintenance in the cerebrospinal fluid but may be essential for establishment or maintenance of a disseminated model of infection.

\textbf{Necrotic Factors}

Necrotic factors are vehicles of virulence because they allow the fungus to overcome structural barriers that the human host uses to prevent invasive infection. Most necrotic factors are enzymes. Because the majority of fungal pathogens are opportunists, these enzymes may have evolved for saprophytic purposes and might be considered nutritional factors, but it seems more likely that their major role in infection is degradation of host tissue. Cellular and tissue damage at the site of the organism are characteristic of many fungal infections. Among the factors that are thought to contribute to this damage are extracellular degradative enzymes, such as proteinases, phosphatases, and DNAses. The evidence adduced for the role of these enzymes is usually diminished virulence in a mutant lacking the activity. Although such findings are persuasive, it is important to bear in mind that a failure to demonstrate involvement of a particular activity is not conclusive because many of these genes are members of families, and several of the products may be involved in pathogenesis.

The earliest identification of a potential necrotic factor was the extracellular proteinase of \textit{C. albicans}, first identified by Staib (143). Further work, largely by Ruchel and coworkers, showed that this enzyme was usually found at infection sites (122, 123). Because this was the first identifiable potential virulence factor, it has been the subject of much investigation and has been a dominant influence on thinking about fungal pathogenesis. Several different names were originally given to the gene for this enzyme, such as \textit{Opal} (104) and \textit{EPR} (65), but all correspond to members of the secreted aspartyl proteinase (\textit{SAP}) gene family. Early experiments from two laboratories provided evidence for the essential role of this activity in infection. McDonald & Odds showed that a variant of \textit{C. albicans} lacking the activity was avirulent in a mouse model (92), and Kwon-Chung et al., using a different variant, repeated the results and found that revertants had regained their virulence (81). Recent studies by Hube and coworkers have shown that there are at least seven and probably nine different genes in the \textit{SAP} gene family; therefore the earlier experiments are hard to interpret, because neither MacDonald nor Kwon-Chung could have had a true structural gene mutant. It
is possible that both Sap variants were blocked in secretion, not synthesis, of an active proteinase.

Experiments designed to study the role of SAP in virulence illustrate the problems associated with analysis of gene families. Of the SAP family members that have been studied, two (SAP1 and SAP3) are regulated during phenotypic switching between the white and opaque forms of WO-1. SAP2 is dominant in the yeast form. The SAP4, SAP5, and SAP6 genes are observed only at neutral pH during the serum-induced yeast-to-hyphal transition (70). The group of Sap4p, Sap5p, and Sap6p isoenzymes plays an important role in the process of induction of SAP2 and thus in the normal progression of systemic infection by C. albicans (126). Hence, the various members of the SAP gene family may have distinct roles in the colonization and invasion of the host. SAP multigene families also exist in other Candida species, such as Candida tropicalis, Candida parapsilosis, and Candida guillermondii (101).

A mutant lacking all nine SAP genes has not been isolated, but the role of several genes has been deduced using individual gene mutants. Six of the SAP genes have been disrupted. Single disruptions of SAP1, SAP2, or SAP3 each caused diminished virulence (71), whereas a triple disruption of SAP4-6, which seem to be coexpressed, also diminished but did not ablate virulence (126). These experiments cannot be considered wholly conclusive, because in neither case did the investigators test the virulence of the disruptant in which the missing gene(s) was replaced.

A proteinase associated with C. immitis may function in the compartmentalization of the spherules into endospores, and the endospores have proteinase activity associated with them. This activity may function to break down lung tissue, the primary site of initial infection, and allow dissemination of the fungus. Cole et al and Yu et al have isolated the genes for a proteinase and a urease, both of which may be important in pathogenesis (29, 168).

A. fumigatus secretes at least two proteinases, both of which are active on elastin, a protein that constitutes about 30% of lung tissue. One of these elastases is a serine proteinase; the other is a metalloproteinase. Both have been cloned; the serine proteinase was disrupted independently in two laboratories, yielding a mutant with no detectable extracellular elastase activity but comparable to the wild type in virulence in mice (100, 135, 152). A third laboratory, using chemical mutagenesis, reported that loss of the activity led to diminished virulence in an immunosuppressed mouse model (77). Whether the difference in these results is due to the method of mutant construction or the model of infection has not been resolved.

C. albicans is known to secrete phospholipases and to possess a phospholipase gene family. Extracellular phospholipases have a role in the pathogenicity of C. albicans, as blood isolates produce significantly more extracellular phospholipase activity than do commensal strains (72). Two different phospholipase B molecules are found in culture supernatants, and the gene for each has been cloned. Disruptants of one gene (PLB1) are less virulent than the parent strain (88). It is obvious
that such enzymes ought to be important in breaching the membranes of host cells during infection. Based on Southern blots, homologues of the \textit{PLB1} gene may also be present in \textit{Candida parapsilosis}, \textit{Candida tropicalis}, and \textit{Candida krusei}, but not in \textit{Candida pseudotropicalis}, \textit{Candida glabrata}, or \textit{S. cerevisiae} (49). However, \textit{S. cerevisiae} has several enzymes with the same activity; therefore presence of the activity alone does not predispose to virulence (85, 165).

\textbf{C. neoformans}, \textit{A. fumigatus}, and \textit{Aspergillus flavus} secrete phospholipases. Several of the corresponding genes have been cloned (37).

\section*{Morphology}

\textbf{Morphological Versatility} Almost all pathogenic fungi can grow in more than one form. \textit{Aspergillus} species, which are classical filamentous molds, form conidia that are the infectious agent. The major exception is \textit{C. neoformans}, which apparently exists only in the yeast form in vivo. In vitro it also grows mostly as a yeast; however, it does form filaments during the mating process, and the small basidiospores have been proposed to be the agents of infection. Furthermore, there is a strong correlation between MAT\textalpha{} (mating-type alpha) and virulence in \textit{Cryptococcus}, and MAT\textalpha{} cells, in contrast to MAT\textalpha{} cells, can undergo haploid fruiting, which involves formation of filaments and basidia, in the absence of a sexual partner (24, 170).

\textbf{Specific Parasitic Cell Forms} \textit{H. capsulatum}, \textit{Blastomyces dermatitidis}, and several (but not all) species of \textit{Candida} can grow both as yeasts and as hyphae. In \textit{C. albicans}, both the yeast and hyphal forms are found at the site of infection, whereas in \textit{Histoplasma} and \textit{Blastomyces}, the yeast form seems to be the major, if not the exclusive, parasitic form. The transition to the parasitic form in \textit{H. capsulatum} leads to a specific pattern of gene expression; this pattern facilitates many of the steps important in infection, including blocking acidification of the phagolysosome and synthesis of the calcium-binding protein, Cbp1p. In contrast to \textit{C. albicans}, the \textit{H. capsulatum} hypha seems to play no role in vivo.

The classical example of a primary pathogen with a specialized parasitic form is \textit{C. immitis}, a soil fungus found in the dry southwest of the United States, especially New Mexico, Arizona, Texas, parts of Utah and Nevada, and the central San Joaquin Valley of California. The saprophytic phase grows in the soil as a septated mycelium in which alternate cells in a hypha undergo cytolysis while their neighbors grow heavy walls. The intact cells are called arthroconidia; they break away from the lysed cells and are disseminated by wind. Arthroconidia are inhaled by mammalian hosts and swell in the lungs to form large multinucleate cells called spherules. The spherules are large, 20–100 \textmu{}m, and segment internally. The nuclei are separated into individual compartments and walled off, forming endospores. Eventually the spherule ruptures, releasing hundreds of endospores, each capable of becoming a new spherule. \textit{C. immitis} is an organism of extreme virulence in part because of the sheer numbers of organisms that can be produced in an infection. Because
there is no genetic system available for this fungus, relatively little is known about
the factors required for pathogenesis. Because spherule formation seems to be
necessary for pathogenesis, any necessary gene whose product is required for
that part of the life cycle should be considered a virulence factor. In fact, a β-1,3-glucanase, whose gene has been cloned, may be important for the transition
from spherule to endospores (78). However, in the absence of a way to create
strains lacking these proteins in order to test for virulence, the importance of these
activities remains unproven.

**MULTIPLE PARASITIC CELL FORMS**  Dimorphic fungi regulate their cellular mor-
phology in response to environmental conditions. For example, ellipsoidal single
cells of *C. albicans* (blastospores) predominate in rich media, whereas filaments
composed of elongated cells attached end-to-end form in response to starvation,
serum, and other conditions. A variety of environmental changes, including a shift
from an aerobic to a fermentative metabolism or growth on particular compounds
such as N-acetyl glucosamine, cause *C. albicans* to switch from yeast to filament-
tous growth. This change is accompanied by changes in carbohydrate metabolism
and an interruption of electron transfer within the cell (82). Both temperature (a
shift to 37°C) and pH can regulate *C. albicans* dimorphism (19). The complex
regulation of these pathways is reviewed in Brown & Gow (18).

The formation of filaments in *C. albicans* has been intensively studied in the
past few years. There are several pathways that control the cell morphology of this
fungus. Two of these are signal transduction pathways culminating in transcrip-
tion factors with orthologues (genes of similar sequence with similar functions)
in *S. cerevisiae*. The gene *CPH1* in *C. albicans* corresponds to *STE12* in *S. cere-
visiae*, and *EFG1* is similar to *PHD1*. The pathway culminating in *CPH1* is so
similar to that of *S. cerevisiae* that several of the *C. albicans* genes complement
the corresponding *Saccharomyces* mutants. Lo et al have presented evidence for
the importance of these last two filamentation pathways by showing that mutants
of *C. albicans* that were unable to form hyphae because they were deleted for both
the transcription factors were avirulent in a mouse model (89). However, such
mutants can form filaments in a gnotobiotic (germ-free) pig infection model and
under certain in vitro conditions (118). Deletions in *INT1* (51) and *CEK1* (31),
both of which block filamentation under some conditions, diminish virulence. In
summary, the evidence suggests that all the morphogenetic pathways in *Candida*
play a role in virulence; however, it seems likely that no one pathway is indispens-
able. Recent virulence studies of filamentation regulatory mutants argue that both
yeast and filamentous forms have roles in infection (99).

Several antifungal agents have morphological effects on *C. albicans*. Amphot-
tericin B prevents filamentous growth (84). In the presence of theazole antifungals,
cells appear as aggregates or chains of swollen structures, many of them with a
bud-like formation (59). Pneumocandins can cause profound changes in hyphal
growth; light micrographs show abnormally swollen germ tubes, highly branched
hyphal tips, and many cells with distended balloon shapes. Electron micrographs
of *Aspergillus* demonstrate that lipopeptides produce changes in cell walls; drug-treated germlings show stubby growth with thick walls and a conspicuous dark outer layer that is much thicker in the subapical regions. The rest of the hyphal tip ultrastructure is unaffected, indicating considerable specificity of the drug for the primary target. The drug-induced growth alteration produced compact clumps in broth dilution wells, making it possible to score morphological effects macroscopically (79).

Many molds form conidia, or vegetative spores; scattered by wind or water, these small, resistant cells serve as a mode of dissemination. In the case of aspergillosis, conidia serve as the propagule that infects debilitated patients. Hydrophobicity is thought to contribute to the efficacy of *Aspergillus* conidia, already an ideal size for deposition into alveoli, to disperse in air. What makes the outermost cell wall layer hydrophobic are interwoven fasicles of clustered proteinaceous microfibrils called rodlets. A rodletless mutant of *A. fumigatus* is hydrophilic and has lost the ability to disperse conidia, but has not lost morbidity in a murine model of invasive pulmonary aspergillosis (153). Although all humans are constantly exposed to *Aspergillus* spores, the immune system is usually effective at preventing colonization and subsequent infection. Hence, the form of aspergillosis that otherwise healthy people develop is related to allergy. Conidia bind to fibrinogen and laminin, but the binding properties are lost as the conidia germinate and produce hyphae (2, 30, 83, 90). Hence, this cell form may play a role more complicated than that of a simple vector in establishing the infection.

PHENOTYPIC SWITCHING  The capacity of fungi to undergo an epigenetic change (regulation of expression of gene activity without alteration of genetic structure) in colony morphology has come to be called phenotypic switching. This phenomenon was often observed in *C. albicans*, but its significance was not understood until Soll’s lab rediscovered it and set out to analyze its role in pathogenesis. Phenotypic switching is characterized by a reversible change, usually occurring between $10^{-3}$ and $10^{-5}$ per cell division, in some property or properties of the cell. Slutsky et al found that colonies arising from cells plated on Lee’s medium showed a standard (smooth) phenotype in 99.99% of the cases, but the remaining 0.01% had a wrinkled surface. Careful observation showed that there were a number of potential colony phenotypes and that the cells could pass from any one of these phenotypes to any other (133). A second paper described a particular strain, WO-1, that has two major colony phenotypes, “white” and “opaque” (134). White colonies almost exclusively contain the classical yeast cells. Opaque colonies contain cells that are bean shaped and differ from white cells in a variety of ways, including surface properties, gene expression, and temperature sensitivity (1, 58, 73). Opaque cells switch to white cells within one generation at 37°C (9, 119). The white phenotype is more virulent in a systemic mouse model of infection, whereas opaque cells are more effective in colonization in a mouse cutaneous infection model (80). The white-opaque transition is relatively rare among *C. albicans* isolates; therefore its overall significance in disease is questionable (103).
Colony phenotype switching is much more common. The various colony forms probably result from different distributions of yeast, pseudohyphal, and hyphal cells in parts of the colony. Cells of different colony phenotypes show different adhesion properties and levels of hypha formation when tested in vitro (156). Strains isolated from patients often show high levels of switching, but the significance of the phenomenon in disease is unclear (137). *C. tropicalis* is capable of high-frequency switching of colony morphology just as *C. albicans* is, and there is more than one strain-specific switching repertoire in *C. tropicalis* (138).

Colony phenotype and genetic similarity, when assessed by comparing groups of commensal and pathogenic strains of *C. albicans* collected from the oral cavities of individuals, demonstrate minor variant colony morphologies (other than smooth) among 38% of pathogenic and 16% of commensal isolates. Commensal and pathogenic groups in the same geographical locale have common clonal origins (63).

*C. neoformans* can also undergo phenotypic switching. A smooth (S) avirulent variant gave rise to wrinkled (WR) and pseudohyphal (PH) phenotypes; they varied in cell shape. Virulence varied in the order WR > PH > S (47).

*H. capsulatum* is an intracellular parasite that can survive in the phagolysosome. The organism is capable of generating variants with diminished β-glucan in the cell wall. Such variants survive in the phagolysosome, but they do not kill the cells like their virulent parents. They are thought to contribute to latency and, much later, to be capable of reactivation (44).

### Adaptations of Specific Organisms

#### Growth at Different pHs

*C. albicans* can grow both at acid and at basic pHs, a reflection of its ability to colonize several niches, ranging from the acid vagina to the neutral oropharyngeal tract. This ability to tolerate a wide pH range is important in several models of virulence. *C. albicans* has two pH-regulated genes, *PHR2* and *PHR1*, the former expressed at acid pH and the latter at neutral and basic pH (106, 127). Deletion of one or the other leads to an inability to grow at the pH at which the gene is expressed. DeBernardis and coworkers showed that virulence of the disruptants was related to the niche: The *PHR2* deletion was avirulent in the vagina, whereas the *PHR1* deletion showed reduced virulence in a systemic model (34). Davis et al showed that deletions of the *RIM101* gene, a pH-regulated transcription factor, also reduced virulence in a systemic mouse model and in an endothelial-cell–damage model, and an activated allele of the *RIM101* gene restored virulence (33).

#### Toxin Production

*A. fumigatus* produces gliotoxin, but it is not known whether clinically significant amounts of gliotoxin are produced in human disease. Gliotoxin inhibits macrophage phagocytosis via DNA fragmentation and apoptosis, targets the neutrophil respiratory burst, and inhibits T-cell activation and proliferation (166, 167). Filamentous fungi also produce several ribotoxins, alpha-sarcin,
restrictocin, and mitogillin, but the biological function of these potent toxins is unknown (94).

**MELANIN** Melanins are scavengers of reactive oxygen intermediaries, making organisms relatively resistant to leukocyte attack (20). Melanin synthesis is catalyzed by a membrane-bound phenoloxidase with a substrate specificity for phenolic compounds that contain hydroxyl or amino groups, such as L-DOPA and dopamine (117). Melanin is deposited in the cell wall of *C. neoformans* (40, 108). The brown color of this pigment can be revealed by growth on birdseed agar or by the Masson-Fontana stain (74). Although much attention has been focused on the role of melanin in cryptococcosis, this substance is constitutively produced in the dematiaceous fungi, such as *Cladosporium* and *Wangiella dermatitidis*. The role of melanin in *W. dermatitidis* has been extensively examined. Melanin seems to be important in initiating an infection, because melanin-deficient strains are much less infective on a colony-forming–unit basis but can cause the same neurological symptoms once infection is established (37–39).

**IRON AND CALCIUM** Iron is an essential element for the growth and metabolism of fungi. *C. neoformans* capsular polysaccharide synthesis is increased by limitation of ferric iron (157). Most pathogenic microbes elaborate siderophores (molecules that can bind iron) to mobilize iron from ferric ligands. One such siderophore, deferoxamine, which is used as an iron chelator in medical practice, has a known association with the development of zygomycete infections because zygomycetes use the iron in deferoxamine efficiently, unlike *C. albicans* or *A. fumigatus* (13, 14).

Hereditary or acquired hemochromatosis, the human disease with progressive iron overload leading to fibrosis and organ failure, poses an increased risk for the development of fungal infection even in the absence of chelator usage. Multiple transfusions have led to severe acquired hemochromatosis followed by the development of zygomycosis (54, 97). Over the past decade *Stachybotrys*, a fungus almost never isolated from human pathologic material, was associated with infants having acute pulmonary hemorrhage and hemosiderosis in households with major water damage (158, 159).

The phagolysosome, in which *H. capsulatum* survives, is a calcium-poor environment. The yeast but not the hyphal phase of this fungus secretes a calcium-binding protein (CBP). This protein aids in the uptake of Ca$^{2+}$ (7). Disruption of CBP leads to avirulence both in vitro in cultured macrophages and in vivo in a mouse pulmonary colonization model (131a).

**SURFACE PROPERTIES** The best known of the fungal immunoevasion systems is the capsule of *C. neoformans*. A viscous polysaccharide capsule composed of glucuronoxyomannan and other components, the capsule is believed to present a surface not recognized by phagocytes, downregulate cytokine secretion, inhibit leukocyte accumulation, induce suppressive T-cells, inhibit antigen presentation, and inhibit lymphoproliferation. Hence, it serves as a barrier to host defenses in a
variety of ways (93, 116). Its importance as a virulence factor is demonstrated by a comprehensive series of analyses of acapsular mutants (21–24).

The opportunistic fungus *Pneumocystis carinii*, an obligate parasite most closely related to *Schizosaccharomyces pombe*, can vary its surface glycoproteins in a manner closely analogous to *Trypanosoma brucei*. A large number of nonexpressed structural genes for the surface glycoproteins exist; any of these can move to an expression site by recombination. Because the genes are highly diverse, replacement in the expression site changes the antigenicity of the surface, providing a defense against host antibodies (160–163).

**MATING TYPE** Virulence in *C. neoformans* is associated with the MATα genotype. Most clinical isolates are serotype A, and this serotype seems to exist exclusively in the MATα configuration. In pathogenic isolates of the other three serotypes, B through D, MATα seems to predominate as well. In contrast to *S. cerevisiae*, the MAT locus in *C. neoformans* contains the gene for the transcription factor *STE12*. Its orthologue in *S. cerevisiae* is required for the hormonal response leading to mating as well as for pseudohyphal growth (124). Recently two conflicting reports about the mechanism of the MAT effect have appeared. Yue et al have found that a disruptant of *STE12α* in serotype A is able to mate but not to undergo haploid fruiting and that the disruptant is normally virulent (170). In contrast, Chang and coworkers have shown that this transcription factor controls the expression of several virulence factors, including melanin and capsule formation in serotype D cells (24). *C. neoformans* serotype D cells deleted for *STE12α* are therefore avirulent, and replacement of the gene restores virulence. Such cells are still able to mate. The apparent contradictions in these two reports may be due to the difference in serotypes; one of the disruptions may have left some function intact, or one of the disruptions may have removed an adjacent gene. The results using serotype D suggest that some parts of the sexual apparatus may be adapted in pathogenic fungi for purposes related to virulence, an interesting idea because many of these fungi seem either to lack a sexual cycle or to mate rarely (24).

**THE HOST**

Commensal fungi are virtually ubiquitous among humans. A yeast microflora is acquired at or soon after birth and persists in many patients despite occasional or routine use of antifungal agents. However, the composition of the flora can change. Patients with AIDS have replacement of original commensal strains of *Candida* species early in the manifestation of the disease (129). Strain replacement occurs from women with vulvovaginal *C. albicans* infections to their male partners or vice versa (130, 131), and infecting strains do not represent a group genetically distinguishable from vaginal commensal isolates (130). The infecting strain can exhibit minor genetic changes in each successive episode of *Candida* vaginitis; therefore they are not genetically stable (131).
Immunocompetence

**ESTROGEN-BINDING PROTEINS**  Estrogen-binding proteins may represent virulence factors for those fungal organisms where men are more likely than women to experience disseminated infection. Exploratory work in this area has concentrated on the organism *Paracoccidioides brasiliensis*, where overt disease following puberty is almost exclusively found among men (146). Specifically, 17β-estradiol demonstrates its protective effect by inhibiting the phase transition from mycelial to yeast forms (90). High-affinity, estrogen-binding sites are sensitive to temperatures above 37°C in yeast-form *P. brasiliensis* cytosol (147). Despite these data, animal models have failed to consistently replicate this disease imbalance in women.

Disseminated infection with *C. immitis* is more common among men and non-white persons, particularly those of Filipino ancestry (113). However, pregnant women with coccidioidomycosis develop dissemination and serious disease more frequently than does the general population (3). The relative resistance of women to disseminated infection reverses because 17β-estradiol, which increases exponentially during pregnancy, stimulates coccidioidal growth in a dose-dependent manner by accelerating the rate of spherule maturation and endospore release (41).

**UNDERLYING DISEASE**  Patients with diabetes mellitus are prone to infection (154) owing to immune system aberrations such as impaired opsonization and decreased chemotactic activity of neutrophils and monocytes. Fungal infections that are common among diabetics include mucosal candidiasis syndromes and, among patients with ketoacidosis, zygomycete infections and perhaps coccidioidomycosis (164). However, diabetics do not appear to be at substantial risk for dissemination. Although vaginal colonization with *Candida* organisms was thought to be more frequent among diabetic women, recent evidence indicates that the ~20% rate of vaginal *C. albicans* carriage is not higher in diabetic women than in nondiabetic women (121). Uncontrolled diabetes predisposes to symptomatic vaginitis (8).

Pregnant women have an increased susceptibility to vaginal infection by *Candida*, resulting in both a higher prevalence of colonization and a higher rate of symptomatic vaginitis. High levels of reproductive hormones increase the glycogen content in the vaginal tissue, providing an excellent carbon source for *Candida* organisms (95). Estrogen may also enhance adherence of yeast cells to vaginal mucosa. The rate of symptomatic vaginitis is maximally increased in the third trimester (105). Symptomatic vaginitis recurrences are more common during pregnancy (35), and relapse episodes following antifungal therapy are common during pregnancy (25).

Protein calorie malnutrition is a risk factor for gastrointestinal zygomycosis (87, 102, 144). Approximately one third of all reported cases of gastrointestinal zygomycosis occur among children (102).

Burn wound victims showed an overall 10-fold increase in fungal infections between 1964 and 1970, probably owing to the introduction of topical antibacterial
agents (107). The notable obvious risks are the elimination of bacterial symbiotic organisms and the breach in cutaneous integrity. Also implicated in risk are the persistent hyperglycemia and glucosuria that develop during a condition called “burn stress pseudodiabetes” (145).

IATROGENIC FACTORS Some studies have shown an increase in either vaginal colonization or symptomatic vaginitis episodes with *Candida* following the use of high-estrogen dose oral contraceptives (55, 139). Other studies have found carriage of yeasts to be increased among those individuals using intrauterine devices (114), diaphragm (66), and nonoxynol 9 (6, 66) as contraception.

Broad-spectrum antibiotic use predisposes to yeast vaginitis by eliminating the normal symbiosis between bacterial and yeast flora, with lower numbers of lactobacilli documented in vaginal cultures among women with symptomatic yeast vaginitis (4, 112). Lactobacilli are thought to maintain a protective effect by steric interference for receptor sites of vaginal epithelial cells and by competing for nutrients (136).

Infection Routes

ENDOGENOUS *C. albicans* is part of the normal human body flora, and it can become pathogenic if it moves from the body compartment where it performs its function as a normal commensal to a compartment that reacts to its presence. Other *Candida* species may become the major endogenous gut yeast when immunocompromised individuals have received antifungal therapy that wipes out *C. albicans*. A breakdown of gut mucosa, from oncologic chemotherapy, radiation, trauma, concurrent viral ulcers, etc., allows a commensal *Candida* strain to relocate from the gut to the bloodstream. Overgrowth of the strain at nonsterile commensal sites such as in the mouth (thrush), esophagus, colon, pulmonary tree, etc., may cause a localized infection that serves more as an irritant for the individual with the problem rather than as a life-threatening illness.

EXOGENOUS Fungi that are environmental saprobes can cause invasive disease in humans if they enter the human body. Usually such organisms are carried in the air, inhaled into the pulmonary tree, and begin a localized invasive infection that may or may not disseminate further in the body. Examples include the primary pathogen *C. immitis* among immunocompetent people, or the opportunistic pathogens *A. fumigatus* or *Pneumocystis carinii* among immunocompromised people. Dissemination can occur either as contiguous extension (such as when sinus infection erodes back through bone into the brain) or by hematogenous spread once the organism erodes into the blood vessels. Some fungi, such as the primary pathogen *Sporothrix schenckii* or the opportunistic molds *Aspergillus, Fusarium*, and the zygomycetes, can be directly inoculated through intact skin as a route of invasion. Yet a third but rare portal of entry into the body for fungal organisms is through ingestion. It is well known that malnutrition is a risk factor for
zygomycete infection, particularly in third world countries, and *Aspergillus* disease of the intestines has led to fatal bleeding from the gastrointestinal tract among heavily immunocompromised humans. For this reason, health food supplements made from natural substances that are not quality-controlled to remove mold spores are contraindicated among immunocompromised patients.

**Immune and Other Host Defenses**

After a microorganism penetrates one of the primary protective surface barriers (intact skin, mucous membranes, and pulmonary system) into the body, it encounters the immune system, which provides defense against invasion of colonizing yeast flora or exogenously inhaled fungal organisms. Humoral immunity (antibodies, complement cascade) and cell-mediated immunity (neutrophils, macrophages/monocytes, eosinophils, basophils, B- and T-lymphocytes, NK cells, and cytokines) are the fundamental areas of host defense.

**HUMORAL IMMUNITY** The humoral immune system consists of soluble substances circulating in the blood. These can include neutralizing antibodies, the complement cascade, additional opsonic proteins (lipopolysaccharide-binding protein, mannose-binding protein), and inflammatory mediators. Antibody-mediated immune responses have at least a partial role in defense against cryptococcosis and candidiasis (61, 169). Phagocytosis experiments in the presence of plasma treated in various ways to inhibit the complement pathways demonstrate that optimal association of *A. fumigatus* conidia with neutrophils is dependent on an active alternative complement pathway (148).

**CELL-MEDIATED IMMUNITY** Cells that destroy infectious agents through phagocytosis or cytotoxicity provide cell-mediated immunity. Cytokines, soluble mediators secreted by some of these cells to regulate the proliferation, maturation, differentiation, and activation of other cells, are also considered part of this arm of the immune system.

The neutrophil is an important phagocytic cell in the defense against fungal infections. Although it is an abundant cell within the circulation, its lifespan of 6–10 h leads to constant replacement of this cell by the bone marrow. The risk of infection in patients is directly related to the quantity and quality of circulating neutrophils. For aspergillosis in particular, the risk of infection increases if the duration of neutropenia (neutrophil count below $0.5 \times 10^9$ cells/liter) is greater than 21 days (56), although the risk of invasive tissue–mold infection and/or fungemia is a continuum, with a greater risk developing as the duration and severity of neutropenia progresses.

Part of the importance of neutrophils relates to the expression of a number of receptors on their cell surface. Glycoproteins (L-selectin, CD11a/CD18, and CD11b/CD18) mediate the adhesion of neutrophils to endothelium in the vascular cell wall. Neutrophils then migrate through the endothelium (diapedesis) into
surrounding tissues, where the processes of chemotaxis and phagocytosis allow destruction of ingested fungi. The elastolytic proteinase of *A. fumigatus* appears to act as an inhibitory agent in vitro to the respiratory burst and chemotaxis activities in a concentration-dependent manner in the human neutrophil (62). *Aspergillus* conidia are effective inducers of host chemokine responses that play a central role in the recruitment of neutrophils into the lung (128).

Macrophages are longer-lived phagocytes than neutrophils. At a site of infection, they appear several hours after neutrophils, persist at the site longer, and can contribute to chronic granulomatous responses (with lymphocytes). Host tissue damage is more extensive with macrophages. Macrophage phagocytosis is not dependent on complement. Interferon gamma increases the ability of tissue macrophages to kill fungi (27, 28, 91). Degradation of phagocytosed microorganisms is carried out in the macrophage by at least two mechanisms: killing by active oxygen intermediates and degradation by lysosomal enzymes. The enzymes have pH optima in the acidic range, and the phagolysosome is highly acidic. The first step in *H. capsulatum* infection is phagocytosis by macrophages. The organism survives intracellularly first by failing to trigger the oxidative burst. The mechanism of this avoidance procedure is not known (42). Second, by preventing host acidification of the lysosome, the fungus avoids the degradative action of the enzymes, because they are much less active at neutral pH (43).

The monocyte is a circulating mononuclear phagocyte that matures into a macrophage in tissues. Under resting conditions, monocytes and macrophages kill ingested microorganisms by a variety of inflammatory mechanisms, but this microbicidal capacity is relatively limited when compared with neutrophils. Human peripheral blood monocytes produce and release the potent inflammatory molecule tumor necrosis factor in a dose-dependent response when exposed to *C. albicans* and other *Candida* yeasts (5). Tumor necrosis factor stimulates neutrophil-induced damage of *A. fumigatus* hyphae (120). In models of myeloperoxidase deficiency and chronic granulomatous disease, myeloperoxidase-independent oxidative and nonoxidative mechanisms appear to be active in hyphal damage by monocytes (36).

T-lymphocytes, named for their site of maturation in the thymus, are central cells in lymphocyte-activated killing. To be recognized by a T-lymphocyte, foreign (fungal) antigens must be presented to the T-lymphocyte by antigen-presenting cells, and both the T-lymphocyte and the antigen-presenting cell must have identical genes for certain major histocompatibility complex proteins so the cell can distinguish self from nonself. Suppressed T-lymphocyte function can lead to fungal infections such as aspergillosis pneumonia, *Pneumocystis carinii* pneumonia, or cryptococcal meningitis.

The brain contains microglial cells, sessile mononuclear phagocytes, that participate in host response to infections. Microglia respond to practically all pathologic events in the central nervous system, aided by infiltrating hematogenous macrophages. Human microglial cells are potent effector cells against *C. neoformans* in vitro in the presence of specific antibody. A murine monoclonal antibody to
the capsular glucuronoxylomannan can produce dose-dependent enhancement of \textit{C. neoformans} phagocytosis by microglia (86). An in vitro–generated microglial cell line, transferred intracerebrally into syngeneic immunocompetent mice, resulted in local protection against a lethal dose of \textit{C. albicans} (11).

One strategy for improving resistance to opportunistic pathogens is to upregulate, during the invasion process, host cellular responses that are relevant to host defense mechanisms. Invasion of endothelial cells by \textit{C. albicans} in vitro stimulates the conversion of arachidonic acid into prostaglandins, upregulating the synthesis of endothelial cell cyclooxygenase and increasing the activity of the endothelial cell phospholipase, modulating the leukocyte response at the candida-leukocyte-endothelial cell interface (45). The secretion of these prostaglandins has no effect on the amount of endothelial cell injury induced by \textit{C. albicans}.

**PLATELETS** Platelets have the potential to play an important role in normal host defenses against invasive aspergillosis. Platelets, like neutrophils, attach to cell walls of the hyphal form of \textit{A. fumigatus}. Damage to the cell wall occurs, and defined hyphal surface glycopolypeptides are released. Rapid appearance of the surface antigen CD63 and release of markers of platelet degranulation are evidence for platelet activation during attachment to hyphae (26).

**CLASSES OF INFECTIONS**

**Mucosal**

The mucosal immune system uses lymphocytes localized into three functional compartments, intraepithelial (T-lymphocytes), lamina propria (B-lymphocytes), and Peyer’s patches. These compartments incorporate cells into the epithelial cell layer, the lamina immediately below, and, for Peyer’s patches found only in the gut, loosely organized lymphoid tissue that are able to sample the antigenic milieu of the lumen.

Infections of gut and vaginal mucosal surfaces are usually described by their location. Gastrointestinal infections are commonly referred to as thrush (oropharynx) and esophagitis. The term thrush can be referenced to the latter half of the eighteenth century, when “country people taught us the virtues of the thrush-moss for sore throats” (132).

The in vitro observations of morphological variation in \textit{Candida} are mirrored by the different clinical presentations in mucosal infection, both for the model oral yeast \textit{Candida} as well as other fungi that cause observable infection among immunosuppressed patients. Typical manifestations of oropharyngeal candidiasis among the AIDS population are pseudomembranes (visible colony-forming units on the mucosal surface), erythema (no visible colony-forming units, but enough infection that inflammatory mediators or use of mucosal nutrients by the yeast causes a reddened color change to the mucosal surface), and angular
cheilitis (fissures at the corner of the mouth where yeast has no space to grow visibly).

In non-AIDS–immunosuppressed patients, fungi such as the zygomycetes and *Fusarium, Aspergillus*, and *Trichosporon* species are emerging as pathogens that may invade the oral mucosa during periods of immunosuppression. These fungal infections of the oropharynx can produce a locally invasive leathery plaque (vegetative hyphae from a tangle of true mold hyphae), an eschar, or a chronic or irregular necrotic ulceration including progression to palatal perforation to form oral-antral fistulae (tissue necrosis). The oral mucosa is a frequent site, in approximately 30%–50% of patients, of involvement during histoplasmosis. The oral lesions are commonly confused with squamous cell carcinomas (15). A variety of mucosal surface lesions can be the initial clue to diagnosis among patients having infection with paracoccidioidomycosis, cryptococcosis, blastomycosis, and coccidiodomycosis (98).

*Candida* organisms gain access to the vaginal lumen and secretions predominantly from the adjacent perianal area (10). Longitudinal changes in yeast flora occurred significantly more often in fecal samples than in oral samples, and significantly less often in sites colonized with *C. albicans* than in sites colonized with other species (109).

**Systemic**

An interesting characteristic of tissue-invasive fungal infections is that some progress to systemic infection while others remain localized. In principle, progression is a function of the degree of immunocompromise of the host and the virulence factors produced by the fungus that has come into contact with that host. Although a great deal is known about host responses to the first steps of fungal infection, predicting whether a patient, with a predefined set of immune defect(s), will contain a localized infection is still so difficult that immediate antifungal treatment is initiated. Some of these treatments are exaggerated and perhaps unnecessary. Further understanding of interactions between the parasite and the host in this situation might allow for more informed and evidence-based decisions regarding diagnostic and therapeutic strategies.

The most common sites of localized nonmucosal fungal infection are the skin with its underlying soft tissues and the lung. Identifying the cause of these infections can be difficult, because superficial cultures from nonsterile sites (surface swab of infected wound, nose culture during sinusitis, sputum culture for an infection deep in the lungs) rarely discriminate between pathogens, commensal flora, and environmental saprobes. Deep cultures from these nonsterile sites (swab following debridement of an infected wound, aspiration of pus from an air-fluid level in the sinuses, bronchoalveolar lavage for pneumonia) are a better basis upon which to treat with antifungal agents, although the uncertainty of pathogenicity is still present. Culture and histologic examination of sterile tissues, such as skin punch biopsy and open-lung biopsy specimens, are helpful in diagnosis.
When infection progresses from localized tissue involvement to widespread disease, recognition of the infecting organism may not be any easier than for contained infections. Disseminated hematogenous yeast fungemias are typically diagnosed by examination of blood samples. Disease may also manifest with embolic skin lesions in the setting of negative blood cultures. These fungal lesions require examination by staining the roof of a blister or by examining a fungal stain of a biopsy specimen while awaiting culture results. Embolic infections result from a plug of organisms or an infected blood clot that occludes blood vessels. When this occurs in the skin layers of the dermis and subcutis, an ischemic tissue cone with necrotic tissue forms above. Therefore, a skin biopsy for a suspected fungal lesion should be taken from the center of the lesion and go deep in order to reach subcutaneous fat.

CONCLUSION

As this review demonstrates, the study of fungal pathogenesis is complex and rapidly evolving. Because properties of the pathogen and of the host seem approximately equally important, and because multiple factors on both sides are involved, the evolving genomic studies of the fungi and of the host have the greatest promise of providing rapid understanding. Work on one gene or one property, no matter how detailed and careful, will not be useful as the kind of comprehensive analysis now available in humans as well as in several pathogenic fungi. Thus the global approach will probably be the most fruitful way to study fungal pathogenesis in the future.

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LITERATURE CITED


Candida albicans treated with three different concentrations of natamycin (Pimafucin) for 6 days. Acta Obstet. Gynecol. Scand. 61:325–28


60. Gow NA, Perera TH, Sherwood-Higham J, Gooday GW, Gregory DW,


136. Sobel JD, Myers PG, Kaye D, Levison


157. Vartivarian SE, Anaissie EJ, Cowart RE,


