# Fungi, mycological disease and pathogenic determinants

*Malcolm Richardson, Michael Ellis*

***The rapid evolution of human fungal infections is providing a strong impetus for understanding pathogenesis and host–fungus interactions and hence new diagnostics.***

The last 20 years have seen unprecedented changes in the pattern of fungal infection in humans. These infections have assumed a much greater importance because of their increasing incidence in debilitated or immunocompromised patients and as a result of major changes in medical practice, increasing international travel and misuse of antimicrobial agents (Warnock and Richardson, 1991). For the first time, an increasing therapeutic armamentarium is developing.

## FUNGI AS HUMAN PATHOGENS

Fewer than 200 of the 200 000 species of fungi described are associated with human disease. These organisms are free-living, independent survivors in nature. Usually fungal infections of humans originate from an exogenous environment, acquired through inhalation, ingestion or traumatic implantation.

A handful of fungi are capable of causing significant disease in otherwise normal individuals. Many more are only able to produce disease under unusual circumstances, mostly involving host debilitation. However, as a result of the numerous developments in modern medicine, these hitherto innocuous organisms have gained increasing prominence as aetiological agents of disease. Any fungus capable of growing at the temperature of the host (37°C) and surviving in a lowered oxidation–reduction state (a situation found in damaged tissue) must now be regarded as a potential human pathogen.

Fungal infections can be classified into a number of broad groups according to the initial site of infection. Grouping the diseases in this manner brings out clearly the degree of parasitic adaptation of the different groups of fungi and the way in which the site affected is related to the route by which the fungus enters the host. This symposium will focus on systemic fungal infections.

## THE SYSTEMIC MYCOSES

These are infections that usually originate in the lungs, but may spread to many other organs. These infections are most commonly acquired as a result of inhaling spores of organisms that grow as saprotrophs in soil or decomposing organic matter, or as pathogens on plants.

Two distinct fungal disease groups exist. The true pathogens comprise a handful of organisms, such as *Histoplasma capsulatum* and *Coccidioides immitis*, capable of invading tissues of normal hosts. These organisms possess unique morphological features contributing to their survival within the host. Clinical disease is characteristically mild, of short duration, with post-recovery immunity or rarely a chronic granulomatous response. However, these true pathogenic fungi also produce life-threatening, unresponsive, relapsing disease in the immunocompromised host (ICH). Thus histoplasmosis and coccidioidomycosis are acquired immunodeficiency syndrome (AIDS)-defining illnesses in North and South America, endemic geographical areas for these fungi.

The second group, the opportunists, consists of less virulent and less well-adapted organisms, such as *Aspergillus fumigatus*, that are only able to invade the tissues of an ICH.

Although new species of fungi are regularly being identified as causes of disease in immunocompromised patients, four diseases still account for most reported infections: aspergillosis, candidosis, cryptococcosis and mucormycosis (zygomycosis).

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## THE CHANGING PATTERN OF FUNGAL INFECTION

Over the past few years, improvements in the management of debilitated medical and surgical patients have led to an unwelcome increase in the number of life-threatening infections caused by true pathogenic and opportunistic fungi (Cornwell et al, 1995; Ellis, 1997; Flanagan and Barnes, 1998; Kappe, 1997; Kuttin, 1997; LaRocco and Burgert, 1997; Paterson and Singh, 1999; Vogeser et al, 1999). These infections are being seen in ever-increasing numbers among patients with cancer, those who have had transplants, people who are taking broad spectrum antibiotics, those on parenteral nutrition, drug addicts and those with AIDS. Estimates of the incidence of these infections are thought to be quite conservative in comparison with their true magnitude, because many fungal infections go undiagnosed.

Some fungi, hitherto regarded as harmless saprotrophs, are being reported as the cause of serious or lethal infection in immunocompromised individuals (Schell, 1995). For instance, *Trichosporon beigelii*, the aetiological agent of the mild dermatological condition white piedra, is now well-documented as a cause of lethal disseminated infection in neutropenic cancer patients and bone marrow transplant recipients (Gueho et al, 1994). *Scedosporium* spp. and *Fusarium* spp. are commonly found in the soil and can cause life-threatening infections that mimic aspergillosis (Boutati and Anaissie, 1997; Westerman et al, 1999). Both *T. beigelii* and *Scedosporium apiospermum* are often resistant to amphotericin B.

Other changes include rising azole resistance in candidosis and emergence of non-albicans *Candida* which are often resistant to commonly used antifungals.

## IMPLICATIONS FOR DIAGNOSIS AND MANAGEMENT

The rise in prevalence of fungal infections has resulted in a need for improved diagnostics. The diagnosis of fungal disease is based upon a combination of clinical observation and laboratory investigation (Richardson and Kokki, 1999).

Laboratory methods for the diagnosis of fungal infection continue to be updated, but depend for the most part on isolation of the fungus in culture, on its detection in clinical material by direct microscopic examination, and on the detection of an immunological response to the pathogen or some other marker of its presence, such as a metabolic product. The traditional morphological approach to mould identification, however, remains the mainstay of diagnosis.

Molecular biological techniques have been developed for the detection of fungal pathogens. Species-specific primers have been designed for a number of organisms, including *A. fumigatus*, *Candida albicans* and *C. neoformans*, and several have been used in attempts to detect fungal DNA in specimens such as blood, tissue, urine and cerebrospinal fluid, following amplification by the polymerase chain reaction.

Monoclonal antibodies to structural components of the major fungal pathogens of humans have the potential to form the basis of new tests for identification of organisms. Their introduction has stimulated significant developments in the diagnosis of fungal infection, by providing improved tests for the detection of circulating fungal antigens in immunocompromised patients, e.g. deep candidosis, aspergillosis or cryptococcosis.

The increased prevalence of life-threatening fungal infection has stimulated interest in the development of new antifungal drugs. New agents, such as the triazoles and the allylamines, have been introduced and new formulations of older compounds, such as lipid-based forms of amphotericin B, have appeared. These developments have improved the treatment of many forms of fungal infection, but problems remain. There are still important infections, such as mucormycosis, for which no reliable treatment has been developed, and many strains of the unusual organisms, such as *C. krusei* and *T. beigelii*, that are now being isolated from debilitated patients are insensitive to current antifungal compounds.

## PATHOGENICITY AND VIRULENCE

The existing definitions of microbial pathogenicity and virulence do not encompass the contributions of both pathogen and host. New definitions have been proposed which are applicable to fungi and the patients they infect (Casadevall and Pirofski, 1999) (*Table 1*).

**TABLE 1.**
**Definitions**

| | |
|---|---|
| Pathogen | A fungus capable of causing host damage; the definition can encompass classical fungal pathogens and opportunistic species; host damage can result from either direct fungal action or the host immune response |
| Pathogenicity | The capacity of a fungus to cause damage in a host |
| Virulence | The relative capacity of a microbe to cause damage in a host |
| Virulence factor (or determinant) | A component of the fungus that damages the host |

adapted from Casadevall and Pirofski (1999)

A pathogenic fungus has several attributes, to successfully invade and survive the host's internal environment:

- The ability to adhere to the stratum corneum or mucous surfaces
- The ability to penetrate host tissues and gain access to target organs or body fluids
- The ability to multiply in vivo. This requires the fungus to be thermotolerant and to adapt to the physiochemical conditions of the host
- The ability to avoid host defence mechanisms
- The ability to damage host tissue. Even if the previous criteria have been fulfilled, a fungus cannot be regarded as pathogenic if it is not able to cause damage to the host.

Therefore, there is little doubt that virulence mechanisms assist in the development of disease. However, disease occurs as a result of host–fungus interactions.

**Adherence**

This is necessary for saprophytic colonization and precedes tissue penetration. Dermatophyte hyphae adhere to the corneocytes of the stratum corneum (*Figure 1*). *A. fumigatus* conidia adhere to mucous surfaces of the respiratory tract. *C. albicans* blastoconidia attach to corneocytes of the stratum corneum and epithelial cells of the mouth, urinary tract and gut mucosa, and endothelial cells of blood vessels (*Figure 2*). It is possible that adhesins are necessary for candidal infection, but adhesive-negative strains need to be raised to prove this.

**Tissue invasion**

Structures that enable pathogenic fungi to penetrate host tissues are found in few zooparasitic fungi. Some human fungal pathogens undergo morphological transformation, from a filamentous to a yeast form of growth, when they invade tissues. In contrast, *Candida* spp. may develop pseudohyphae or true hyphae when they invade (*Figure 3*). Hyphae are necessary for the invasion of the hard keratin of human hair by dermatophyte species (*Figure 4*). Environmental temperature, pH, serum factors and nutrition, may induce hyphal transition in vitro. Investigation of the genetic basis of polymorphism in *C. albicans* has provided basic molecular biological characteristics of this organism, including the initial characterization of putative transcription factors that affect hyphal formation (Kobayashi and Cutler, 1998). Additionally, strains with higher proteolytic and phospholipase activity have been shown to be more virulent and specific enzyme-deficient mutants of *C. albicans* are less pathogenic (Ghannoum, 2000).

The ability of *A. fumigatus* to transform from a saprophyte to a parasite and invade living tissue is dependent upon a number of virulence

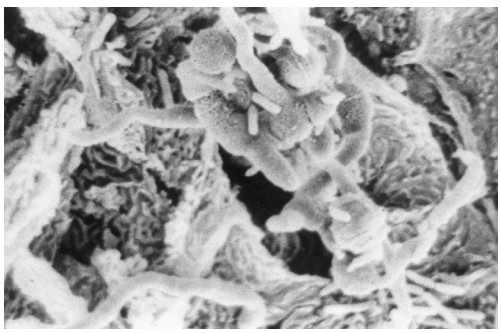

*Figure 1. Adherence of arthroconidia and hyphae of* Trichophyton mentagrophytes *to human stratum corneum.*

*Figure 3. Histopathological section of human larynx showing invasion by hyphae of* Candida albicans.

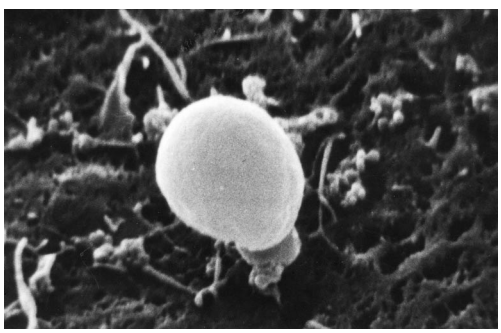

*Figure 2. Adherence of* Candida albicans *to a monolayer of human gastrointestinal tract epithelial cells.*

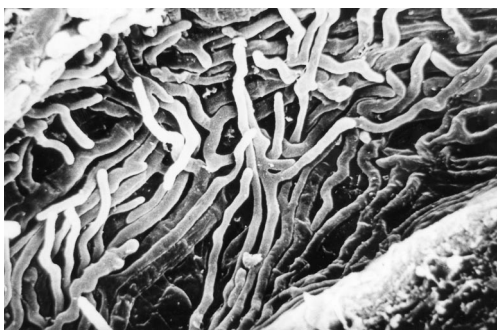

*Figure 4. Invasion of human hair shaft by hyphae of* Trichophyton mentagrophytes.

attributes (reviewed in Latge, 1999), including the production of secreted proteases and elastases. Extensive invasion of lung tissue by *A. fumigatus* has suggested a key role for fungal proteases during infection (*Figure 5*). *A. fumigatus* appears to have an ecological niche in the bronchi, because the nutrient and temperature conditions are favourable to its saprophytic growth. This may explain the discrepancy between the relatively low incidence of *Aspergillus* among all the fungal airborne spores and its more frequent isolation from the bronchopulmonary tree.

**Multiplication within tissue**

The morphological variations (dimorphism) seen in pathogenic fungi are phenotypic modifications enabling fungi to survive host defences and to multiply in host tissues. There are no morphological advantages for penetration of host cells, apart from penetration of epithelial cells by *C. albicans* pseudohyphae. Having survived the non-specific defence mechanisms that operate during the initial contact with a human host and having established a focal point of tissue invasion, a fungal pathogen has to assure itself of a continued existence by multiplication and dissemination to target organs and specific tissues.

**Tissue damage**

The successful completion of the pathogenic process by fungi includes damage to tissue, accompanied by a characteristic inflammatory response (*Figure 6*).

Fungal antigenic or biochemical composition elicit host responses but how specific these are is unclear. No one tissue change seems to be entirely characteristic or pathognomonic of fungal disease. Suppuration, macrophages, giant cells, caseous necrosis and fibrosis are variably seen components. Chronic suppuration with fibrosis is the most general tissue change in deep fungal infection. The polymorphonuclear leucocyte is usually the primary reacting cell, but occasionally it may be the macrophage or giant cell.

Fatal infection is uncommon among individuals with intact immunological defence mechanisms. The infections that occur in such persons are caused by a restricted number of pathogenic fungi. In immunocompromised patients, however, the mechanisms that protect the host from infection are deficient or absent, and this appears to be the principal reason for the increased incidence of fungal infections with pathogenic as well as the common saprophytes among such individuals.

Persistence of the fungus in the host, regardless of the host's immune status, may be the result of perturbation or evasion of the immune response by mechanisms as yet unknown, but not by direct fungal toxicity. It is evident that the cell wall of pathogenic fungi, for instance *C. albicans* is central to this aspect of immunomodulation.

*Aspergillus* spp., unlike many other fungal species, can colonize the respiratory mucosa, even in the normal airway, sensitizing and causing allergic bronchopulmonary aspergillosis. More commonly, the fungus colonizes damaged airways, such as in patients with cavitary lung disease. The mechanism for this is poorly understood. *A. fumigatus* slows ciliary beat frequency concurrently damaging respiratory epithelium in vitro. *A. fumigatus* produces a number of biologically active substances including gliotoxin which slow ciliary beating and damage epithelium, which may influence invasion of the airways.

Taking high-dose steroids is a risk factor for invasive aspergillosis. Corticosteroids can suppress human neutrophil- or elutriated monocyte-induced hyphal damage in a dose-dependent manner and increase the growth rate of *Aspergillus* by 40%.

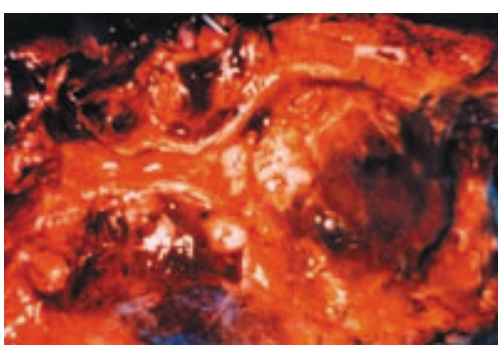

***Figure 5. Extensive invasion by* Aspergillus fumigatus *of human bronchus.***

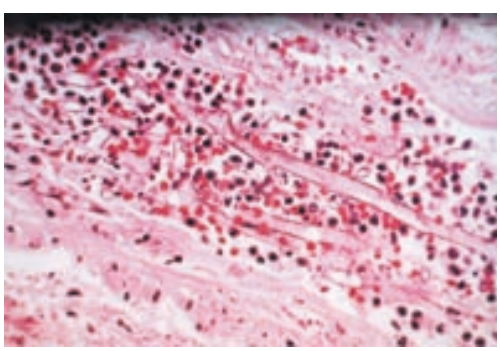

***Figure 6. Histopathological section of human palate showing extensive tissue damage caused by* Rhizopus *species and a pronounced inflammatory response.***

## HOST RESPONSES

### Aspergillosis

Host defence to invasive aspergillosis is primarily an innate mechanism. Elucidation of the host defence mechanisms in aspergillosis has been the focus of many studies in recent years (Latge, 1999; Roilides et al, 1998; Romani and Howard, 1995).

Conidia of *A. fumigatus* bypass the upper respiratory tract defences, because of their small size (<4 μm) and aerodynamic properties, reaching distal lung regions. Here the host defences rely on phagocytic cells to remove conidia.

Our knowledge of the role that phagocytic cells play in the eradication of *A. fumigatus* has been advanced only recently. The published studies on the ability of phagocytic cells to kill conidia of *A. fumigatus* have produced conflicting results, largely as a result of the many different in vitro assays of phagocytosis and intracellular killing being used. However, it is well recognized that defective neutrophil function, e.g. in chronic granulomatous disease or cytotoxic-induced prolonged neutropenia, is a key factor in pathogenesis of invasive aspergillosis.

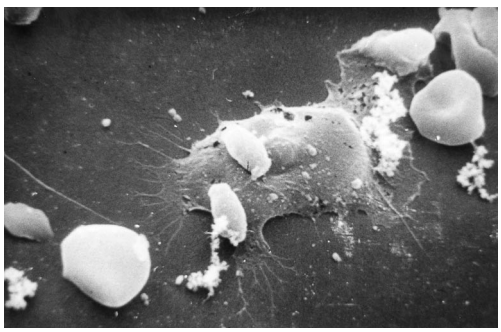

***Figure 7. Binding of* Candida albicans *to a human neutrophil immediately before phagocytosis.***

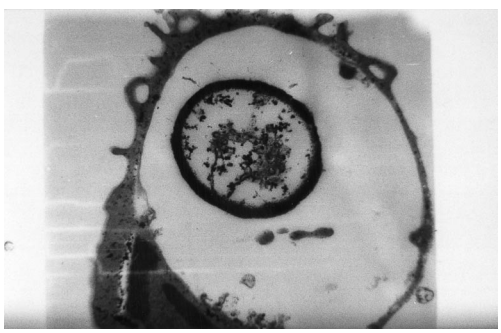

***Figure 8. Intracellular killing of* Candida albicans *by a human neutrophil.***

### Candidosis

It has long been known that neutrophils are important effector cells against *C. albicans* (*Figures 7*, *8*). However, it is becoming clear that neutrophils may be regarded not only as terminally differentiated effector cells, but also capable of synthesizing immunomodulatory cytokines (Romani and Howard, 1995). The ability to release cytokines confers upon these cells an important, previously unexpected role in shaping the subsequent immune responses. **HM**

## KEY POINTS

- Hitherto innocuous fungi now demonstrate their capability for lethal invasive disease.
- Innovative molecular diagnostics are proving useful in this regard.
- Knowledge of host–fungus interactions is advancing but are incompletely understood.

*Conflict of interest: none.*


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