Candidiasis and cryptococcosis of humans

Steve Smith explores the world of pathogenic fungi

A number of fungal species have the capacity to exploit human beings, either giving rise to merely uncomfortable circumstances or life threatening conditions of disseminated infection. Foremost amongst such fungi are the yeasts *Candida* and *Cryptococcus*. The former are more common, while the latter appear to have all the attributes required to colonise and exploit if not destroy the human race, yet their occurrence remains relatively infrequent.

The overall incidence of fungal infections has increased dramatically over the last 20 years according to recent survey data. Infections due to *Candida* species account for approximately 80% of all major systemic fungal infections and are the most common fungal infections of the immunocompromised. Furthermore *Candida* species are the second most common cause of urinary tract infections and the fourth most common cause of nosocomial blood stream infections, which in turn are associated with considerable mortality.

Potentially pathogenic *Candida* species exist on a worldwide basis (Table 1) of which *Candida albicans* is the most common. However incidence of the non -*Candida albicans* species (NACs), such as *Candida tropicalis* and *Candida glaberata*, appears to be on the increase. Although frequently present as part of an individuals natural or commensal microbial flora, they should also be considered as opportunistic and highly dangerous pathogens.

Studies have attempted to relate the different *Candida* species of Table 1 to a range of underlying diseases or medical practices, however, while failing to make appropriate connections, such studies have underscored the great variety of circumstances or patient states which *Candida* can exploit.

Medical procedures and associated practice continue to encourage *Candida* infection or candidiasis (Table 2), however, underlying disease is a major if not the major predisposing factor in candidiasis. The advent of human immunodeficiency (HIV) gave rise to heightened interest in fungal infections, hence greater focus on the development of more efficacious patient care and support. Individuals from the developed world with acquired immunodifficiency syndrome (AIDS) so frequently present with candidiasis that the presence of *Candida* in forms like oral and oesophageal thrush (Fig. 1) became part of the diagnostic criteria of the AIDS syndrome. In such circumstances *Candida* can occur on its own or with other infectious entities for example Herpes Simplex Virus (HSV) in a form of "symbiotic" relationship exploiting the ulcers produced by HSV.

All *Candida* species have similar morphology and physiology. In common with insects and arthropods chitin/n-acetyl glucosamine is a major structural component, incorporated into cell walls in association with considerable quantities of mannose and mannose moieties (Fig. 2). Surface exposure of such mannose moieties has marked consequences in the interaction of pathogen and human host as mannose and mannoproteins have a variety of roles including adhesion to living and inanimate surfaces, cellular recognition by both innate and adaptive vertebrate immunity and the modulation of adaptive immune responses by *Candida*. Fig. 2 also clearly demonstrates the dimorphic nature of *Candida* species, which are capable of both filamentous (hyphal/psuedohyphal) and unicellular (blastospore) forms of growth. Such an ability, which may be included in a list of *Candida* virulence factors (Table 3), remains something of a mystery. Many reports and investigations have attempted to elucidate the factors, which control or influence *Candida* growth forms, resulting in authorities citing temperature, pH shifts and partial gas pressures amongst others. In common with such contradictory findings only speculation exists as to the perceived value of a dimorphic growth habit. A hyphal form mediates greater anchorage, a consequence of greater surface area contact, in turn enhancing penetration of host structural entities, particularly when assisted by localised hyphal-tip growth and lytic enzyme release. Contrastingly as single or budding cells *Candida* is readily dispersed by circulation of body fluids ensuring effective host exploitation thereby giving rise to disseminated or systemic infection. However, authorities recognise that *Candida* in unicellular form can compromise and penetrate host structural entities, furthermore experimental evaluation and control of dimorphism remains problematical obscuring our understanding of any merits associated with a dimorphic growth habit.

Peripheral parts of the human body are readily colonised by *Candida* (Fig.1) and although uncomfortable for the individual are generally not life threatening. Systemic or disseminated *Candida* may develop from such sources, however in the absence of tissue trauma (Fig. 3), a main route of *Candida* entry is the gastro-intestinal (GI) tract. Initial events in establishment and subsequent penetration of the GI tract are for the fungus fraught. Regular passage of food solids, mucus movement and the sloughing of epithelial cells make for a most unstable site, potential precluding adhesion and adherence.

The early sequence of events in establishment and penetration of the GI tract by *Candida* in a unicellular form include, contact with the mucus blanket, penetration of the mucus blanket, establishment and attachment to underlying epithelial cells. As *Candida* cells are immobile, contact with overlying mucus comes through natural churning of gut contents. Penetration of overlying mucus may occur through pores present in the gel matrix, hydrolytic enzyme action or a combination of both. Attachment to epithelial cells is best summarised as a two stage process mediated by complex physico-chemical and biological forces. Initially a loose or reversible adhesion occurs, mediated by van de Waals forces, cell surface charge and cellular hydrophobicity. Tight or irreversible adhesion follows, mediated by the likes of adhesins, which in the case of *Candida* are mainly mannoproteins. Experimental animal models show *Candida* attachment to the surface of the smaller intestine in approximately 20 minutes, further penetration of the epithelium assisted by hydrolytic enzyme release and potential dissemination may occur within three hours. Although hydrolytic proteases play an important role in compromising host defences, phospholipases should also be considered amongst

Table 1 Species commonly causing invasive Candidiasis

Organism	Comments	Incidence
Candida albicans	Most opportunistic fungal infection of the Candidas. First reported in 1853	>50%
Candida tropicalis	Most common Candida species in India. First reported in 1910	15 - 30%
Candida glabarata	Shows marked resistance to fluconazole	15 - 40%
Candida parapsilosis	Infections associated with invasive devices. First reported in 1917	15 - 30%
Candida krusei	Shows some resistance to fluconazole	10 - 35%

Other human pathogen Candida species include: *Candida lusitaniae, Candida guilliermondii, Candida kefyr, Candida rugosa, Candida dubliniensis, Candida zyelanoides*

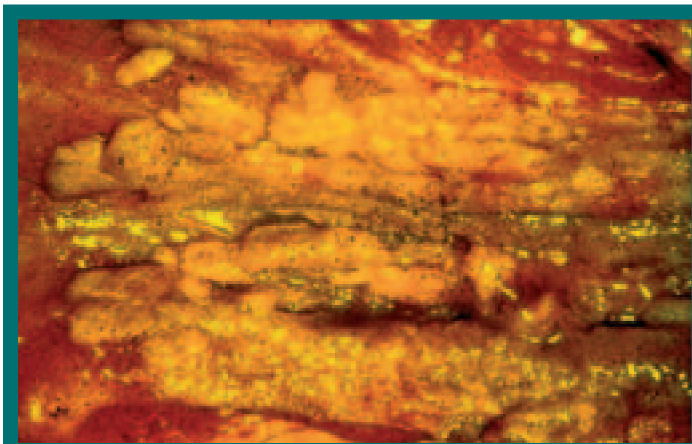

Fig.1: *Candida oesophageal overgrowth*

Table 2 Predisposing factors to Candidiasis

- Pre-existing disease state: patients with HIV/AIDS, maligant tumours*, leukaemia and diabetes.
- Immunosuppressed patients through chemotherapy*, and graft (marrow, kidney, liver) transplantation.
- Catheterization: central venuous cathethers*, bladder catheters
- Intravenous drug abuse
- Burns: certain reports indicate 60% of burns/dressings associated with *Candida* presence
- Antibiotic/imprudent antibiotic usage*

***many of these states and conditions interrelate and interact**

Candida virulence factors. Tissue invasion and haemolysis by *Candida* strains is associated with an ability to produce phospholipases, enzymes capable of disrupting the phosphate component of fatty acids, thereby compromising membrane integrity and function.

Candida has the capacity to survive certain host strictures like low iron levels, sometimes termed nutritional immunity, through production of iron-chelating agents and possible exposure of membrane proteins required to internalise said chelating agents. However elements of both innate and adaptive vertebrate immune systems readily target the mannose component of *Candida* cell walls. Unimpaired human immune systems, through a complex series of interactions potentially leading to phagocytosis by the likes of macrophages, effectively destroy invading *Candida* cells. In contrast, those with impaired immune systems particularly T-cell function, are at great risk of mortality through the likes of septicaemia, as some estimates consider *Candida* infection to be the cause of death in 20% of AIDS patients.

In common with susceptibility to *Candida*, the immunocompromised are at grave risk of cryptococcosis, infections mediated by the yeast *Cryptococcus*. This yeast is not restricted to humans alone and can be isolated from a variety of domestic and wild animals. Rare amongst yeasts, this organism shares taxonomic features common to large and edible fungi like mushrooms, which some consider to be the most sophisticated of fungi. Although 38 species of *Cryptococcus* have been isolated and characterised, only one species of two strains or subspecies namely *Cryptococcus neoformans vr neoformans* and *Cryptococcus neoformans vr gattii* are capable of human infection. Incidence of cryptococcosis within the global human population, in common with other fungal infections, is hard to quantify, due to poor monitoring particularly in developing countries. Within developed countries, where adequate monitoring exists, it is nowhere near as common as candidiasis. US public health reports from the CDC indicate that in the 1980s there were on average only 730 cases per annum across the whole of the USA.

As an opportunistic pathogen in the mould of *Candida*, a number of predisposing factors including pre-existing disease state or therapeutic practice promote *Cryptococcus* incidence. Infection rates of 2.8% have been reported for organ transplant recipients of the developed world of which 42% have died. Under such circumstances continued low incidence of *Cryptococcus* must be an abiding wish, as once *Cryptococcus* is established the outcome for those infected appears grim. However, as medical practitioners embrace ever more technologies and esoteric protocols, associated with the likes of organ transplantation and cancer chemotherapy, the numbers of immunocompromised individuals increase so *Cryptococcus neoformans* infection rates will inexorably rise.

From the 1980s cryptococcal infection rates increased in US HIV patients to levels of 5-10% of all AIDS sufferers. Rates vary on a global basis, in Europe the % frequency amongst AIDS patients is somewhat lower, contrastingly what reliable data exists from Africa indicates 30% cryptococcal

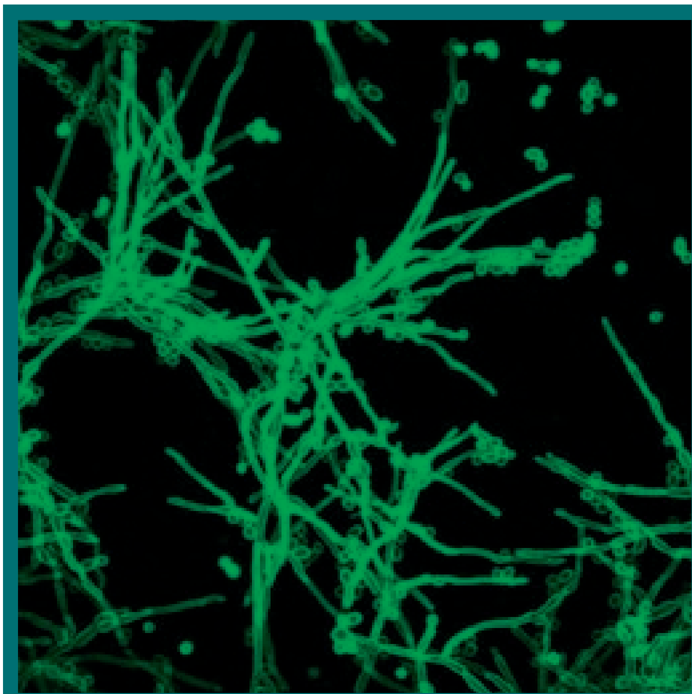

Fig. 2: *Filamentous and unicellular forms of Candida albicans complexed with mannose specific FITC-lectin*

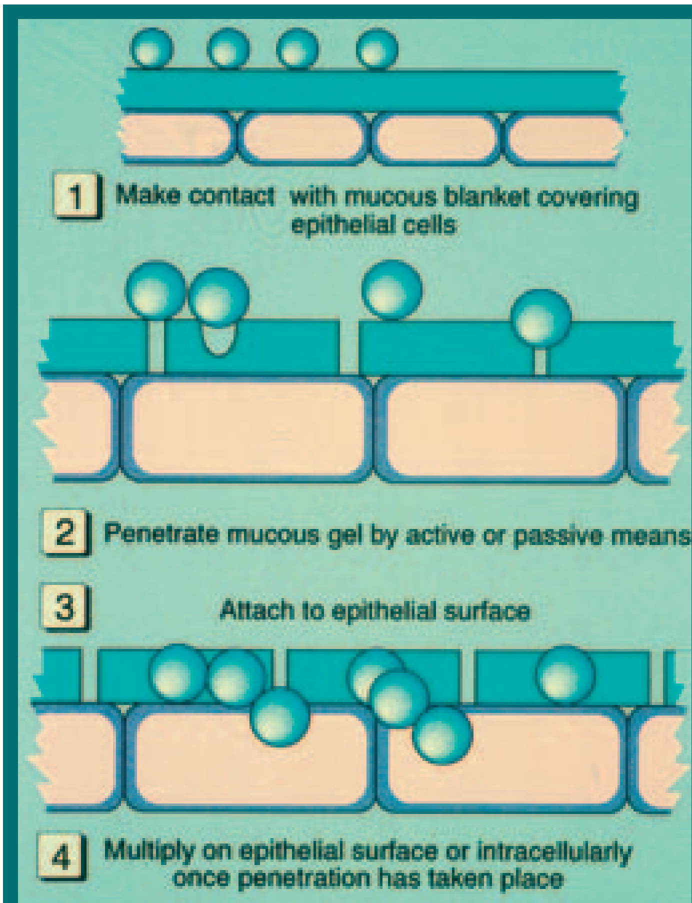

Fig. 3: *Diagrammatic outline of Candida penetration of the GI tract*

infection amongst AIDS patients, who may only survive for two weeks without suitable antifungal treatment. Antifungals can ameliorate infection and extend patient longevity however *Cryptococcus* remains the 4th most common cause of death amongst AIDS patients.

Many cases of cryptococcosis are not diagnosed until signs of meningitis appear. The lungs are commonly considered an initial site of infection through inhalation of yeast cells or dust contaminated with such cells giving rise to a primary pulmonary or lymph nodule. Cryptococcal infections like those of *Aspergillus* have been termed "pigeon fanciers lung" as birds and their guano are a common source of infection. Levels of viable *Cryptococcus* cells in pigeon faeces can reach 50 million cells per gram guano. Furthermore a study has shown that cryptococcal cells remain viable and potentially infectious in dried bird droppings for over 400 days.

The nodule may lie dormant for an indefinite period and frequently resolve in healthy individuals without recourse to treatment. However, in the immunocompromised pulmonary cryptococcosis develops rapidly into disseminated infection in the form of many pulmonary nodules with associated symptoms of fever, malaise, chest pain, dyspnoea and weight loss. Amongst AIDS patients, infection of the brain and the meninges is the most common clinical manifestation of cryptococcosis. Mental abnormalities and changes follow including, drowsiness, confusion, headache, a rise in intercranial pressure, septic shock and death.

From the standpoint of a microbiologist and mycologist *Cryptococcus* appears the ultimate human yeast pathogen, exhibiting many of the attributes theoretically associated with a destructive and robust entity. Table 4 lists the many and potent virulence factors manifest by *Cryptococcus* hence its relatively uncommon nature remains puzzling. While all these factors deserve attention the unique in fungal terms capsule of *Cryptococcus* deserves particular investigation. The capsule is composed of three carbohydrate moieties, upwards of 88% of the capsule is glucuronoxylomannan (GXM) with smaller contributions from galactoxylomannan and mannoprotein. These are large molecular weight but viscous polysaccharides, hence the capsule is continuously shed, resulting in detectible quantities appearing in patients sera and cerebrospinal fluid. Poor affinity amongst lectins and other biological entities, which are specific for mannose, is a defining characteristic of GXM, even though mannose is a major GXM component. Although the capsule may have arisen to protect cells from desiccation or protozoan predation, its presence has marked consequences for those infected by *Cryptococcus*. Important elements of innate vertebrate immunity including mannose binding lectin (MBL) are rendered virtually redundant, furthermore GXM is also poorly immunogenic, hence terms such as "immunological paralysis" have been coined to describe the poor immune response of animal models exposed to *Cryptococcus*. Such is the apparent value of the capsule to *Cryptococcus* one can only speculate why it is such a unique feature amongst yeasts in general.

Pathogenic yeast merit our continued attention and constant vigilance, particularly with ever increasing numbers of immunocompromised patients and the commonplace use of intrusive devices. Furthermore their suppression and control remain problematical as in comparison to numbers of antibacterial therapeutics few efficacious antifungals are available, in part a consequence of the common eukaryote nature of yeast pathogen and human host. Favoured agents such as amphotericin are associated with poor patient tolerance or very expensive when formulated in more tolerated forms while resistance to other major antifungals such as azoles can develop in days. In the light of such circumstances human infection by pathogenic yeast will remain at best problematical and at worst life threatening.

Table 3 Putative members of the *Candida albicans* Virulence Panel

- Rapid switching of expressed phenotype
- Hypha and psuedohyphal formation
- Thigomotropism
- Surface hydrophobicity
- Surface virulence molecules - receptors, adhesins, immunomodulators
- Molecular mimicry - host like surface components
- Lytic enzymes - proteinases, phospholipases and others
- Growth rate
- Undemanding nutrient requirement

Table 4 Virulence factors of *C. neoformans*

- Capsule
- Melanin synthesis
- Resistance to dessication
- Growth at 37°C
- Mannitol synthesis
- Proteinase secretion
- Phospholipase
- Urease

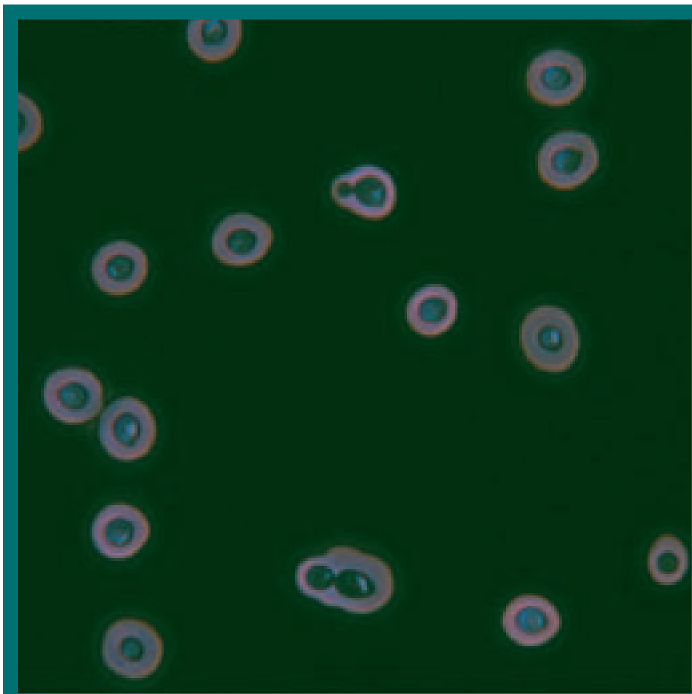

Fig. 4: *Cryptococcus neoformans and associated capsule as visualised by indian ink staining*

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