Pathogenicity determinants of Candida albicans: potential targets for immunotherapy?

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Epidemiology, diagnosis and treatment of candidiasis

Since the early 1960s Candida albicans has emerged from being an infrequent pathogen to become one of the commonest agents of nosocomial infection. In a national surveillance conducted in the USA between 1986 and 1990, Candida species were the fifth most common isolates to be recovered from blood cultures taken from hospital in-patients, and the fourth most commonly recovered pathogen (from all sites) in intensive care units (Edwards, 1991). Candida species are becoming nearly as common a cause of hospital-acquired infection as the more familiar bacterial pathogens. By far the commonest species to cause clinical infection is C. albicans.

In the systemic form of this infection, mortality is far higher than with bacterial septicaemia, being in the order of 38–59% (Komshian et al., 1989; Wey et al., 1988). This is due to difficulties in both diagnosis and treatment. The former is hampered by the fact that the great majority of patients with invasive candidiasis do not demonstrate any of the characteristic clinical features and therefore have to be distinguished from other causes of a pyrexia failing to respond to broad spectrum antibiotics. Blood cultures are unreliable in confirming the diagnosis, being negative in 56% of patients with necropsy-proven disseminated candidiasis (De Repentigny & Reiss, 1984). There has therefore been a great deal of interest in developing more sensitive forms of laboratory diagnosis, including lysis centrifugation and serological tests based on the detection of circulating candidal antigens (Matthews, 1993). The rationale is that if a reliable diagnosis can be made earlier on in the course of the infection this will facilitate prompt treatment with the appropriate antifungal chemotherapy, causing a fall in mortality. But treating fungal infections is also more difficult than treating those caused by bacteria. This is because fungi, like the host, are eukaryotic. Therefore it is intrinsically difficult to find agents with selective toxicity for fungal cells. Amphotericin B is still the agent of choice in life-threatening invasive candida infections but it is highly toxic (Edwards, 1991). In an attempt to reduce toxicity various liposomal preparations of amphotericin B have now become commercially available, but whether this will succeed in reducing nephrotoxicity while retaining efficacy has yet to be the subject of large-scale clinical trials. Amphotericin B is sometimes used in combination with fluconazole.

The other main family of antifungal agents is the azoles, of which fluconazole has proved highly effective in treating mucocutaneous candidiasis. As it is so much better tolerated than amphotericin B there has been understandable interest in using it to treat life-threatening systemic infections. This is a more controversial application, but with the new high-dose regimes its efficacy may be approaching that of amphotericin B. Unfortunately it is not used in conjunction with amphotericin B because of the risk of antagonism. Clinical resistance to fluconazole treatment has also been described in 5–10% of patients with AIDS (Dupont et al., 1994).

In the longer term it is to be hoped that entirely new types of antifungal agents will be developed. Ideally these should be non-toxic so that they can be safely given to patients suspected of having systemic candidiasis before the diagnosis is proven, and able to be used in conjunction with existing antifungals without the risk of antagonism. The latter would help to prevent the emergence of resistance strains and enable the usage of lower doses of antifungal agents while maintaining effectiveness. There are two synergistic approaches to the development of a new generation of antifungal agents. The first involves a better understanding of the pathogenesis of these infections to identify potential targets. The second involves harnessing intrinsic host defence mechanisms against this opportunistic pathogen, which may be a means of selectively inhibiting candidal cells while avoiding damage to host cells. Both approaches will now be discussed, with particular reference to heat-shock proteins as a potential target and human recombinant antibodies as a therapeutic agent.
Table 1. Factors predisposing to candidiasis

<table>
<thead>
<tr>
<th>Common presentations</th>
<th>Typical predisposing factors</th>
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<tr>
<td>Superficial</td>
<td>Antibiotics</td>
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<td>Oral thrush</td>
<td>Pregnancy</td>
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<td>Vaginal thrush</td>
<td>Diabetes</td>
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<td>HIV-infection*</td>
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<td>Impaired granulocyte function</td>
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<td>Immunosuppressive drugs</td>
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<td>Infancy</td>
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<td>Old age</td>
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<td>Trauma</td>
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<td>CMC</td>
<td>Often familial</td>
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<tr>
<td>Chronic mucocutaneous candidiasis</td>
<td>Impairment of cell-mediated immunity associated with</td>
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<td>– multiple superficial sites affected</td>
<td>endocrine disorders</td>
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<tr>
<td>Systemic</td>
<td>Acute leukaemia</td>
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<tr>
<td>Pyrexia unresponsive to</td>
<td>Acute lymphoma</td>
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<td>broad-spectrum antibiotics in</td>
<td>Cytotoxic chemotherapy</td>
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<td>immunosuppressed patient</td>
<td>Corticosteroids</td>
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<td>Broad-spectrum antibiotics</td>
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<td>Organ transplantation</td>
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<td>Intravascular catheters</td>
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<td>Severe burns</td>
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<td>Major gastrointestinal surgery</td>
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* Usually ‘erythematous oral candidosis’, not thrush, in pre-AIDS patients.

Pathogenesis of candidiasis

*C. albicans* is a common commensal of the mouth and the gastrointestinal and vaginal tracts, and is probably harboured by most individuals at some stage in their lives. Nevertheless a wide range of predisposing factors can lead to it becoming invasive (superficially or systemically) and symptomatic (Table 1). Individuals infected with the HIV virus frequently present with oral candidiasis as the initial manifestation. Both oral and oesophageal candidiasis are characteristic features of AIDS, yet systemic candidiasis is surprisingly rare despite severe deficiency in cell-mediated immunity (Sugar, 1994). Only now is systemic candidiasis being seen in very advanced cases of AIDS, in whom profound generalized immunosuppression occurs. Similarly, patients with the rare condition chronic mucocutaneous candidiasis (CMC) with various defects in cell-mediated immunity suffer protracted, multiple, superficial infections but do not succumb to the systemic form of the disease. Primary immunodeficiency states also suggest differences in the host-defence systems involved at different sites of infection (Matthews et al., 1988). Severe primary immunodeficiency syndromes involving T lymphocytes, such as Di George Syndrome and Glanzmann–Riniker Syndrome, are associated with oral candidiasis. Both oral and systemic candidiasis occur in Swiss-type agammaglobulinaemia, with defects in both immunoglobulin production and cell-mediated immunity.

Clearly, then, many different components of the host-defence system are involved in holding *Candida* infections at bay. This is reflected in the many different animal models which have been developed to investigate the relative importance of humoral and cell-mediated immunity, and the conflicting results obtained. It seems most likely that the requirement for innate, humoral or cell-mediated immunity varies with the site of infection and the age, species and even strain of the host. Thus both animal work (Al-Doory, 1970; Cutler, 1976; Rogers et al., 1976; Giger et al., 1978; Mourad & Friedman, 1968; Pearsall et al., 1978; Maiti et al., 1985; Matthews et al., 1991a; Ashman & Papadimitriou, 1993) and clinical observation (Matthews et al., 1988) suggest the importance of humoral immunity in systemic candidiasis, while cell-mediated immunity seems critical in preventing superficial mucocutaneous candidiasis. Different inbred strains of mice vary in their susceptibility to candidiasis. In some strains this can be linked to deficiency in complement but in others the reason for this susceptibility remains obscure (Ashman & Papadimitriou, 1988; Marquis et al., 1986).

The properties which contribute to the ability of *C. albicans* to behave as a pathogen are equally complex. Some of the more well-characterized virulence factors follow.

Adhesion and adhesins

Tissue colonization is an essential prelude to invasion. The ability of *C. albicans* to bind to a variety of host surfaces, including epithelial and endothelial cells, fibrin-platelet matrices, neutrophils, denture acrylic and Teflon,
is clearly a useful asset in the pathogenesis of infection (Rotrosen et al., 1986; Douglas, 1987). Though Candida species more commonly colonize the host without causing symptomatic infection, adhesion is obviously only one of many factors contributing to pathogenicity. Under normal circumstances, competition with the commensal flora reduces the availability of binding sites for Candida.

The virulence of a species of Candida can be correlated with its ability to adhere to various cell types. For example, the most virulent species, C. albicans, is the most adherent to epithelial cells, followed by C. tropicalis and C. parapsilosis. The least virulent species, C. guilliermondii, C. krusei, and C. pseudotropicalis, show relatively little adhesion (King et al., 1980).

The propensity of different strains of C. albicans to spread and cause outbreaks may also be influenced by differences in ability to adhere to various surfaces. Whilst most cases of systemic candidiasis arise as a result of autoinfection, outbreaks due to cross-infection from patient to patient with a single strain are now well-described (Burnie et al., 1985, 1987). These are particularly liable to occur among long-stay patients on adult or neonatal intensive care units, and involve carriage on the hands of nursing staff. One strain causing such an outbreak survived better on hands than did control strains, and this difference was accentuated by washing with a chlorhexidine detergent, the outbreak strain being retained in much larger numbers. This was found to be due more to the increased ability of the outbreak strain to adhere to epithelial cells, than to intrinsic resistance to chlorhexidine (Burnie, 1986).

Several mechanisms underlie the interaction between Candida and the cell surface. Candidal mannanproteins seem to be important adhesins in mediating attachment to buccal and vaginal cells (Douglas, 1992). The protein component of the mannanprotein binds in a lectin-like manner to glycoside receptors on the host cell membrane. Hydrophobic interactions appear to be of primary importance in the adhesion of Candida to plastic surfaces, in vitro, but their involvement in adhesion to epithelial cells is less clear. Fibronectin on the surface of vaginal epithelial cells or in the extracellular matrix can also act as a yeast receptor.

Some adhesins are only expressed on the hyphae or germ-tube forms of C. albicans. This is the case for those binding the complement (C3) conversion products C3d and iC3b (Heidenreich & Dierich, 1985). It was shown that C. albicans and C. stellatoidea, but not less pathogenic Candida species, formed a rosette with erythrocytes coated with iC3b and C3d. A series of monoclonal and polyclonal antibodies against human complement receptor were then examined, one of which bound specifically to C. albicans (Edwards et al., 1986). The C3d receptor was partially purified from C. albicans pseudohyphae by affinity chromatography and characterized as a 60 kDa glycoprotein (Calderone et al., 1988; Linehan et al., 1988). The iC3b receptor on C. albicans shares homology with the alpha-chain of neutrophil receptors for iC3b, as determined by the binding of monoclonal antibodies, and is induced by mycelial transformation and high glucose concentrations (Gilmore et al., 1988). Blockade of these receptors with monoclonal antibodies significantly enhanced phagocytosis of the C. albicans suggesting these receptors promote pathogenicity by inhibiting phagocytosis. However there are some reservations as to whether this does occur in vitro since it seems difficult to repeat the results in the presence of serum (Edwards, 1992). At present the role of complement receptors on Candida in pathogenesis is uncertain.

**Proteinase**

Proteinases are found in the culture media of most of the clinically important Candida species (Ruchel, 1981; MacDonald, 1984). Secretory acid proteinase antigen can be demonstrated in patients infected with C. albicans, as can specific antibodies (Macdonald & Odds, 1980; Ruchel et al., 1988). The proteinase is induced at an early stage and probably facilitates epidermal invasion, being associated with the appearance of cavitations around yeasts in epidermal corneocytes in mice and deposited around the yeasts themselves (Ray & Payne, 1994). More uncertain is whether proteinase is still required once Candida has entered the bloodstream. One paradox is that the enzyme has a very low pH optimum, yet the tissues being invaded normally have a neutral pH. Possibly the rapidly growing C. albicans cells at the infected site lower the pH sufficiently for the proteinase to be active.

After phagocytosis, the proteinase antigen is expressed on the surface of intracellular fungal elements (Borg & Ruchel, 1990). Sometimes ingested blastoconidia survive and germinate, causing death of the phagocyte. This resistance to phagocytosis may be linked to the production of Candida proteinase within the phagolysosome, which could then attack proteins involved in the generation of microbicidal oxygen radicals. It would explain the paradoxically low respiratory response of phagocytes to viable C. albicans (Sasada & Johnston, 1980).

Three independent investigations (Macdonald & Odds, 1983; Kwong-Chung et al., 1985; Ross et al., 1990) have suggested that proteinase-deficient mutant strains are considerably less lethal than the proteolytic parent strain in animal models. Since in each case the mutations were chemically induced, the yeasts might have been afflicted by the mutagenesis in other ways. When the first Candida proteinase gene was cloned and sequenced (Hube et al., 1991), it was hoped that confirmation might come from gene disruption experiments, but now it is known that at least five, and possibly six, genes code for a family of proteinases (Hube et al., 1994). It is very difficult to know which one(s) play a key role in pathogenesis - which are secreted, which are active outside the cell and active at the pH operating in the infected tissue, and how to selectively delete individual proteinase genes.

Another approach has been to examine the effect of a proteinase inhibitor on the course of systemic murine candidiasis (Edison & Manning-Zweerink, 1988; Ruchel...
et al., 1990). The first of these studies failed to demonstrate any benefit. The second showed a relative protective effect if the inhibitor was given both prior to, and repeatedly after, the infection, suggesting a possible role in the early stages of candidiasis. Antibodies inhibiting Candida protease activity have not yet been described. If a suitable specific inhibitor for human use could be produced, its benefit might be limited because the infection is too advanced by the time the patient becomes symptomatic.

**Phenotypic and genetic switching**

*C. albicans* is capable of high-frequency, reversible phenotypic switching, which could help the yeast adapt to its diverse locations as a commensal or opportunistic pathogen. The molecular mechanisms of phenotypic switching have not yet been elucidated. One of the most dramatic examples is that observed with strain WO-1, which switches between a white and an opaque colony-forming unit (Slutsky et al., 1987). This occurred at 25°C on nutrient-poor medium. Other phenotypic traits affected by this switching include cell morphology, lipid and sterol content, adhesion to buccal epithelium, levels of acid protease secretion, antigen expression and susceptibility to a number of antifungal agents (reviewed in Soll, 1992).

Clearly then phenotypic switching does affect a number of putative pathogenicity determinants, but if it is involved in pathogenesis it must occur at the site of infection and generate phenotypes beneficially adapted to different host environments. While switching does appear to occur at the site of infection (Soll et al., 1989) it is more difficult to demonstrate whether or not it actually contributes to the yeast adapting to the commensal or pathogenic state.

Genetic switching of *C. albicans* is readily observed by pulsed-field gel electrophoresis (Rustchenko-Bulgac & Howard, 1993). It can be precipitated by relatively trivial procedures in vitro, and its occurrence was evident within a single isolate, an individual infection and even during the course of an outbreak, the switched isolate being capable of further infection (J. Burnie & N. Khattak, unpublished observation). It may be that the strains which are most prone to genetic switching show greater virulence. The genetic instability of *C. albicans* also raises the question of whether two isolates with different genetic fingerprints could be the same strain which has undergone genetic switching. In practice, most of the genetic typing systems developed for Candida are probably not sensitive enough to be affected by this phenomenon, but it could be a problem for typing systems based on pulsed-field gel electrophoresis.

**Heat-shock protein 90**

Immunoblotting sera from patients with systemic candidiasis identified an immunodominant antigen of 47 kDa (Matthews et al., 1984) which was subsequently identified as a subcomponent of Candida heat-shock protein 90 (hsp 90; Matthews & Burnie, 1989). Heat-shock proteins are major targets for specific immunity in many infections (Young, 1992) and cross-reactivity to shared epitopes on these highly conserved proteins may cross-protect against different microbes (Kaufmann, 1990). Whereas patients who recovered from systemic candidiasis mounted a good antibody response to the 47 kDa subcomponent of hsp 90, fatal cases had little antibody or titres fell. The antigen was demonstrated circulating in infected patients' sera (Matthews et al., 1987), in part as immune complexes (Neale et al., 1987), and formed the basis of a series of diagnostic assays (Matthews & Burnie, 1988; Matthews et al., 1991b). Like *Saccharomyces cerevisiae*, *C. albicans* probably has two structurally similar hsp 90 proteins, one produced constitutively and the other induced by stress such as heat-shock (Matthews, 1991).

Using the Pepscan technique to epitope-map *C. albicans* hsp 90 revealed an epitope, LKVIRK, which was recognized by all patients with antibody to the 47 kDa antigen (Matthews et al., 1991b). This epitope is highly conserved, and is central to the proposed protein-binding site of human hsp 90 (Schwartz & Mizukami, 1991; Sullivan & Toft, 1993). A murine monoclonal antibody raised against this epitope, given prophylactically, reduced mortality in a mouse model of invasive candidiasis (Matthews et al., 1991a). In this case then an autoantibody was beneficial, protecting the host from infection. Cohen (1992) has suggested that the immune response to dominant conserved antigens such as heat-shock proteins is already anticipated by preformed regulatory networks, which channel the autoimmune response down pathways which prevent the development of autoimmune disease. There are multiple benefits for the host in using such targets for specific protective immunity. Being shared between different microbial pathogens, cross-protective immunity is achieved. Repeated exposure to the same epitope or potential pathogens and commensals ensures a high level of specific, high affinity antibody is present early on in the infection, effectively bridging the gap between innate and specific immunity. Since the microbe is not viable without these highly conserved, critical molecules, mutants which would lack the target and therefore be resistant cannot arise. Antibody to LKVIRK has been found to cross-react with Gram-positive bacteria such as *Corynebacterium jeikeium* (Matthews, 1991).

Physiologically, hsp 90 serves a chaperone function, binding to a large variety of cellular proteins to maintain correct protein folding and to prevent premature degradation (Gething & Sambrook, 1992). This suggests a possible mechanism whereby the monoclonal antibody to the binding site of hsp 90 was protective in a murine model of invasive candidiasis (Matthews & Burnie, 1992). If extraneous circulating candidal hsp 90 binds to serum proteins, causing them to malfunction, then an antibody preventing this would be beneficial. If this is indeed the mechanism, then such an antibody would need to be given in conjunction with a chemotherapeutic agent, at least in immunocompromised patients unable to use their own immune system to clear the yeast. The situation would be analogous to giving antibody in conjunction
with antibiotics to treat Gram-negative bacterial septicemias.

**Immunotherapy**

Recent advances in antibody engineering have greatly increased the chances of us being able to use antibody therapy as an effective adjunct to chemotherapy (Table 2; Matthews, 1994). The more traditional approach is to link murine heavy and light chain variable domains from a protective murine monoclonal antibody to human immunoglobulin constant region domains. Unfortunately this still induces a human anti-mouse antibody response, which reduces the clinical effectiveness of the resultant chimeric antibody (Winter & Harris, 1993). This response is lessened by grafting only the hypervariable antigen-binding complementarity-determining regions (CDRs) into the human antibody.

Alternatively the polymerase chain reaction can be used to amplify up the genes encoding heavy and light chain variable domains (Marks et al., 1991; Kang et al., 1991; Soderland et al., 1992). These can be linked together with a suitable spacer fragment as a single chain Fv (scFv) and expressed at the tip of a filamentous phage to produce a phage display library. We have produced such a library into the human antibody. We have isolated epitope-specific human recombinant antibodies which we assessed in a mouse model of invasive candidiasis. Given alone, 2 h after a lethal dose of intravenous *C. albicans*, a scFv against LKVIRK produced a highly statistically significant drop in mortality (R. Matthews and others, unpublished observations).

**Conclusions**

A great deal of investigation has been carried out in order to better define and characterize the pathogenicity determinants of *C. albicans*. Although much further forward, the exact mechanisms of pathogenesis are still unclear. Excluding toxin-producing pathogens, the same is true of many bacterial infections, but for most of these suitable antibiotics are available making the need to understand their pathogenesis less urgent.

<table>
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<tr>
<th><strong>Table 2. Advances in antibody engineering</strong></th>
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<tbody>
<tr>
<td><strong>Type</strong></td>
</tr>
<tr>
<td>Chimeric antibody</td>
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<tr>
<td>'Reshaped' antibody</td>
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<tr>
<td>Human recombinant antibody fragments</td>
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Although a number of pathogenicity determinants have been outlined here, their relative importance in bringing about disease remains unclear. Indeed there are likely to be more, as yet unidentified, virulence factors. The situation may become clearer if we unravel the host-defence mechanisms – which obviously play a key role in combatting *C. albicans* in healthy individuals. By identifying the components of the yeast targeted by the immune response we may be able to mimic this and produce beneficial forms of immunotherapy to supplement the chemotherapeutic agents currently available.

**References**


Pathogenicity determinants of *Candida albicans*


