**REVIEW
ARTICLE****Pathogenicity determinants of
Candida albicans: potential targets for
immunotherapy?**

Ruth C. Matthews

Tel: +44 61 5512. Fax: +44 61 5504.

Department of Medical Microbiology, University of Manchester Medical School, Oxford Road, Manchester M13 9PT, UK

Keywords: *Candida albicans*, pathogenicity determinants, immunotherapy**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 flucytosine.

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

Common presentations		Typical predisposing factors
Superficial	Oral thrush Vaginal thrush	Antibiotics Pregnancy Diabetes HIV-infection* Impaired granulocyte function Immunosuppressive drugs Infancy Old age Trauma
CMC	Chronic mucocutaneous candidiasis – multiple superficial sites affected	Often familial Impairment of cell-mediated immunity associated with endocrine disorders
Systemic	Pyrexia unresponsive to broad-spectrum antibiotics in immunosuppressed patient	Acute leukaemia Acute lymphoma Cytotoxic chemotherapy Corticosteroids Broad-spectrum antibiotics Organ transplantation Intravascular catheters Severe burns Major gastrointestinal surgery

* 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 more 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 mannoproteins seem to be important adhesins in mediating attachment to buccal and vaginal cells (Douglas, 1992). The protein component of the mannoprotein 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 receptors were then examined, one of which bound significantly 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 deter-

mined 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 vivo* 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* proteinase 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 on 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

Table 2. Advances in antibody engineering

Type	Construct
Chimeric antibody	Murine heavy and light chain variable domains, human constant domains
'Reshaped' antibody	Murine hypervariable CDRs grafted into human antibody
Human recombinant antibody fragments	Single chain Fv or Fab fragments amplified from human lymphocytes and expressed in phagemids (combinatorial phage display libraries)

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 from a patient who recovered from invasive candidiasis and had high titre antibody to *Candida* hsp 90. From this we 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.

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

- Al-Doory, Y. (1970).** An immune factor in baboon anti-candida serum. *Sabouraudia* **8**, 41–47.
- Ashman, R. B. & Papadimitriou, J. M. (1988).** Murine candidiasis: strain dependence of host responses after immunization. *Immunol Cell Biol* **66**, 231–237.
- Ashman, R. B. & Papadimitriou, J. M. (1993).** Strain dependence of antibody-mediated protection in murine systemic candidiasis. *J Infect Dis* (letter) **168**, 511–513.
- Borg, M. & Ruchel, R. (1990).** Demonstration of fungal proteinase during phagocytosis of *Candida albicans* and *Candida tropicalis*. *J Med Vet Mycol* **28**, 3–14.
- Burnie, J. P. (1986).** Candida and hands. *J Hosp Infect* **8**, 1–4.
- Burnie, J. P., Odds, F. C., Lee, W., Webster, C. & Williams, J. D. (1985).** Outbreak of systemic *Candida albicans* in intensive care unit caused by cross-infection. *Br Med J* **290**, 746–748.
- Burnie, J. P., Matthews, R. C., Lee, W., Philpott-Howard, J., Brown, R., Damani, N., Breuer, J., Honeywell, K. & Jordans, Z. (1987).** Four outbreaks of nosocomial systemic candidiasis. *Epidemiol Infect* **99**, 201–211.
- Calderone, R. A., Lineham, L., Wadsworth, E. & Sandberg, A. L. (1988).** Identification of C3d receptors of *Candida albicans*. *Infect Immun* **56**, 252–258.
- Cohen, I. R. (1992).** The cognitive paradigm and the immunological homunculus. *Immunol Today* **13**, 490–494.
- Cutler, J. A. (1976).** Acute systemic candidiasis in normal and congenitally thymid-deficient (nude) mice. *J Reticuloendothel Soc* **19**, 121–124.
- De Repentigny, L. & Reiss, E. (1984).** Current trends in immunodiagnosis of candidosis and aspergillosis. *Rev Infect Dis* **6**, 301–312.
- Douglas, L. J. (1987).** Adhesion of *Candida* species to epithelial surfaces. *CRC Crit Rev Microbiol* **15**, 27–43.
- Douglas, L. J. (1992).** Mannoprotein adhesins of *Candida albicans*. In *New Strategies in Fungal Disease*, pp. 34–50. Edited by J. E. Bennett, R. J. Hay & P. K. Peterson. Edinburgh: Churchill Livingstone.
- Dupont, B., Improvisi, L., Ronin, O. & Provost, F. (1994).** Clinical impact of antifungal drug resistance in AIDS patients. *Abstract no. S14.4 ISHAM*. Adelaide, Australia.
- Edison, A. M. & Manning-Zweierink, M. (1988).** Comparison of the extracellular proteinase activity produced by a low-virulence mutant of *Candida albicans* and its wild-type parent. *Infect Immun* **56**, 1388–1390.
- Edwards, J. E. (1991).** Invasive *Candida* infections. Evolution of a fungal pathogen [editorial]. *New Engl J Med* **324**, 1060–1062.
- Edwards, J. E. (1992).** Human complement receptor-like molecules on *Candida*. In *New Strategies in Fungal Disease*, pp. 54–62. Edited by J. E. Bennett, R. J. Hay & P. K. Peterson. Edinburgh: Churchill Livingstone.

- Edwards, J. E., Jr, Gaither, T. A., O'Shea, J. J., Rotrosen, D., Lawley, T. J., Wright, S. A., Frank, M. M. & Green, I. (1986). Expression of specific binding sites on *Candida* with functional and antigenic characteristics of human complement receptors. *J Immunol* **137**, 3577–3583.
- Gething, M.-J. & Sambrook, J. (1992). Protein folding in the cell. *Nature* **355**, 33–45.
- Giger, D. J., Domer, J., Moser, S. & McQuitty, J. (1978). Experimental murine candidiasis: pathological and immune responses in T lymphocyte depleted mice. *Infect Immun* **21**, 729–737.
- Gilmore, B. J., Retsinas, E. M., Lorenz, J. S. & Hostetter, M. K. (1988). An iC3b receptor of *Candida albicans*: structure, function and correlates for pathogenicity. *J Infect Dis* **157**, 38–46.
- Heidenreich, F. & Dierich, M. P. (1985). *Candida albicans* and *Candida stellatoidea* in contrast to other *Candida* species, bind iC3b and C3d but not C3b. *Infect Immun* **50**, 598–600.
- Hube, B., Turver, C. J., Odds, F. C., Eiffert, H., Boulnois, G., Köchel, H. & Ruchel, R. (1991). Sequences of the *Candida albicans* gene encoding the secretory aspartate proteinase. *J Med Vet Mycol* **29**, 129–131.
- Hube, B., Monod, M., Schofield, D. A., Brown, A. J. P. & Gow, N. A. R. (1994). Differential regulation of six genes encoding secretory aspartyl proteinase in *Candida albicans*. Abstract no. P04.6, ISHAM. Adelaide, Australia.
- Kang, A. S., Barbas, C. F., III, Janda, K. D., Benkovic, S. J. & Lerner, R. A. (1991). Linkage of recognition and replication functions by assembling combinatorial antibody Fab libraries along phage surfaces. *Proc Natl Acad Sci USA* **88**, 4363–4366.
- Kaufmann, S. H. E. (1990). Heat shock proteins and the immune response. *Immunol Today* **11**, 129–136.
- King, R. D., Lee, J. C. & Morris, A. L. (1980). Adherence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. *Infect Immun* **27**, 667–674.
- Komshian, S. V., Uwaydah, A. K., Sobel, J. D. & Crane, L. R. (1989). Fungemia caused by *Candida* species and *Torulopsis glabrata* in the hospitalized patient. *Rev Infect Dis* **11**, 379–390.
- Kwong-Chung, K. J., Lehman, D., Good, C. & Magee, P. T. (1985). Genetic evidence for the role of extracellular proteinase in virulence of *Candida albicans*. *Infect Immun* **49**, 571–576.
- Linehan, L., Wadsworth, E. & Calderone, R. (1988). *Candida albicans* C3d receptor, isolated by using a monoclonal antibody. *Infect Immun* **56**, 1981–1986.
- Macdonald, F. (1984). Secretion of inducible proteinase by pathogenic *Candida* species. *Sabouraudia* **22**, 79–82.
- Macdonald, F. & Odds, F. C. (1980). Inducible proteinase of *Candida albicans* in diagnostic serology and in the pathogenesis of systemic candidosis. *J Med Microbiol* **13**, 423–435.
- Macdonald, F. & Odds, F. C. (1983). Virulence for mice of a proteinase-secreting strain of *Candida albicans* and a proteinase-deficient mutant. *J Gen Microbiol* **129**, 431–438.
- Maiti, P. K., Kumar, A., Kumar, R. & Mohapatra, L. N. (1985). Role of antibodies and effect of BCG vaccination in experimental candidiasis in mice. *Mycopathologia* **91**, 79–86.
- Marks, J. D., Hoogenboom, H. R., Bonnert, T. P., McCafferty, J., Griffiths, A. D. & Winter, G. (1991). By-passing immunization. Human antibodies from V-gene libraries displayed on phage. *J Mol Biol* **222**, 581–597.
- Marquis, G., Montplaisir, S., Pelletier, M., Mousseau, A. & Auger, P. (1986). Strain-dependent differences in susceptibility of mice to experimental candidosis. *J Infect Dis* **154**, 906–909.
- Matthews, R. C. (1991). HSP 90, yeasts and *Corynebacterium jeikeium*. *Epidemiol Infect* **107**, 273–283.
- Matthews, R. C. (1993). Early diagnosis of systemic candidal infection. *J Antimicrob Chemother* **31**, 809–812.
- Matthews, R. C. (1994). Recent advances in antibody engineering. *Serodiag Immunother Infect Dis* (in press).
- Matthews, R. C. & Burnie, J. P. (1988). Diagnosis of systemic candidosis by an enzyme-linked dot immunobinding assay for a circulating immunodominant 47 kDa antigen. *J Clin Microbiol* **26**, 459–463.
- Matthews, R. C. & Burnie, J. P. (1989). Cloning of a DNA sequence encoding a major fragment of the 47 kilodalton stress protein homologue of *Candida albicans*. *FEMS Microbiol Lett* **60**, 25–30.
- Matthews, R. C. & Burnie, J. P. (1992). The role of hsp 90 in fungal infection. *Immunol Today* **13**, 345–348.
- Matthews, R. C., Burnie, J. P. & Tabaqchali, S. (1984). Immunoblot analysis of the serological response in systemic candidosis. *Lancet* **ii**, 1415–1418.
- Matthews, R. C., Burnie, J. P. & Tabaqchali, S. (1987). Isolation of immunodominant antigens from the sera of patients with systemic candidosis and characterization of the serological response to *Candida albicans*. *J Clin Microbiol* **25**, 230–237.
- Matthews, R. C., Burnie, J. P., Smith, D., Clark, I., Midgley, J., Conolly, M. & Gazzard, B. (1988). *Candida* and AIDS: evidence for protective antibody. *Lancet* **ii**, 263–266.
- Matthews, R. C., Burnie, J. P., Howat, D., Rowland, T. & Walton, F. (1991a). Autoantibody to HSP 90 can mediate protection against systemic candidosis. *Immunology* **74**, 20–24.
- Matthews, R. C., Burnie, J. P. & Lee, W. (1991b). The application of epitope mapping to the development of a new serological test for systemic candidosis. *J Immunol Methods* **143**, 73–79.
- Mourad, S. & Friedman, L. (1968). Passive immunisation of mice against *Candida albicans*. *Sabouraudia* **6**, 103–105.
- Neale, T. J., Muir, J. C. & Drake, B. (1987). The immunochemical characterisation of circulating immune complex constituents in *Candida albicans* osteomyelitis by isoelectric focusing, immunoblot, and immunoprint. *Aust NZ J Med* **17**, 201–209.
- Pearsall, N. N., Adams, P. L. & Bunni, R. (1978). Immunologic responses to *Candida albicans*. III. Effect of passive transfer of lymphoid cells or serum on murine candidiasis. *J Immunol* **120**, 1176–1180.
- Ray, T. L. & Payne, C. D. (1994). Role of *Candida* acid proteinase in adhesion and invasion of murine epidermis. Abstract no. S5.5, ISHAM. Adelaide, Australia.
- Rogers, T. J., Balish, E. & Manning, D. D. (1976). The role of thymus dependent cell-mediated immunity in resistance to experimental disseminated candidiasis. *J Reticuloendothel Soc* **20**, 291–298.
- Ross, I. K., De Bernadis, F., Emerson, G. W., Cassone, A. & Sullivan, P. A. (1990). The secreted aspartate proteinase of *Candida albicans*: physiology of secretion and virulence of a proteinase-deficient mutant. *J Gen Microbiol* **136**, 687–694.
- Rotrosen, D., Calderone, R. A. & Edwards, J. E. (1986). Adherence of *Candida* species to host tissues and plastic surfaces. *Rev Infect Dis* **8**, 73–85.
- Ruchel, R. (1981). Properties of a purified proteinase from the yeast *Candida albicans*. *Biochim Biophys Acta* **659**, 99–113.
- Ruchel, R., Boning-Stutzer, B. & Mari, A. (1988). A synoptical approach to the diagnosis of candidosis, relying on serological antigen and antibody tests, on culture, and on evaluation of clinical data. *Mycoses* **31**, 87–106.

- Ruchel, R., Ritter, B. & Schaffrinski, M. (1990). Modulation of experimental systemic murine candidosis by intravenous pepstatin. *Zentralbl Bakteriol* **273**, 391–403.
- Rustchenko-Bulgac, E. P. & Howard, D. H. (1993). Multiple chromosomal and phenotypic changes in spontaneous mutants of *Candida albicans*. *J Gen Microbiol* **139**, 1195–1207.
- Sasada, M. & Johnston, R. B. (1980). Macrophage microbicidal activity. Correlation between phagocytosis-associated metabolism and killing of *Candida* by macrophages. *J Exp Med* **152**, 85–98.
- Schwartz, J. A. & Mizukami, H. (1991). A metal-linked gapped zipper model is proposed for the 90 kDa heat shock protein-estrogen-receptor interface. *Med Hypotheses* **35**, 140–145.
- Slutsky, B., Staebell, M., Anderson, J., Risen, L., Pfaller, M. & Soll, D. R. (1987). 'White-opaque transition': a second high-frequency switching system in *Candida albicans*. *J Bacteriol* **169**, 189–197.
- Soderland, E., Simonsson, A. C. & Borrebaeck, C. A. K. (1992). Phage display technology in antibody engineering: design of phagemid vectors and *in vitro* maturation systems. *Immunol Rev* **109**–124.
- Soll, D. R. (1992). Switching and its possible role in *Candida* pathogenesis. In *New Strategies in Fungal Disease*, pp. 156–172. Edited by J. E. Bennett, R. J. Hay & P. K. Peterson. Edinburgh: Churchill Livingstone.
- Soll, D. R., Galask, R., Isley, S., Rao, T. V. G., Stone, D., Hicks, J., Schmid, J., Mac, K. & Hanna, C. (1989). Switching of *Candida albicans* during successive episodes of recurrent vaginitis. *J Clin Microbiol* **27**, 681–690.
- Sugar, A. M. (1994). Candidiasis in AIDS patients. *Abstract no. S3.1, ISHAM*. Adelaide, Australia.
- Sullivan, W. P. & Toft, D. O. (1993). Mutational analysis of hsp 90 binding to the progesterone receptor. *J Biol Chem* **268**, 20373–20379.
- Wey, S. B., Mori, M., Pfaller, M. A., Woolson, R. F. & Wenzel, R. P. (1988). Hospital-acquired candidemia: the attributable mortality and excess length of stay. *Arch Intern Med* **148**, 2642–2645.
- Winter, G. & Harris, W. J. (1993). Humanized antibodies. *Immunol Today* **14**, 243–246.
- Young, D. B. (1992). Heat shock proteins – immunity and auto-immunity. *Curr Opin Immunol* **4**, 396–400.