Drug Discovery Today: Disease Models (2005) 2 (2) : 155-159. http://dx.doi.org/10.1016/j.ddmod.2005.05.013

Genetic Models of Candida Infection and Host Resistance Factors

Robert B. Ashman[1,*], Camile S. Farah[1], Yan Hu[1], Xin Li[1], Christine Wells[2]

[1]School of Dentistry, University of Queensland, Brisbane, Qld, Australia
[2]School of Biomolecular and Biomedical Science, Griffith University, Nathan, Qld, Australia

Abstract

Infections caused by the yeast Candida albicans can occur on the skin, on mucous surfaces, or in disseminated form throughout the body and corresponding models have been developed in vivo. Patterns of infection differ in inbred mice, and the variability has been shown to be genetically regulated. The availability of gene-knockout mice, has also contributed significantly to an understanding of the cellular and molecular basis of the host response.

Introduction

Candida albicans is a ubiquitous yeast that commonly colonizes mucosal surfaces in humans. Normally innocuous, it can cause disease when host resistance is compromised by debility, disease or by administration of drugs or immunosuppressive agents. Under these conditions, it can proliferate on mucosal surfaces such as the oral cavity and vagina, and if introduced into the circulation, can infect most tissues and organs, but particularly the kidney and brain.

The differing manifestations of *Candida* infection in humans have led to the development of a variety of experimental models, addressing both systemic and mucocutaneous candidiasis, and in the latter case, both oral and vaginal disease. Models of the systemic disease are most commonly used, and have been most thoroughly investigated. By contrast, mucocutaneous infections that accurately reflect the human disease have been difficult to establish in mice.

In vivo models

Systemic candidiasis

After intravenous challenge, mice display two different pat-terns of mortality. Death either occurs within the first two weeks, or occurs after a more extended period. The relative resistance of inbred strains appears to be independent of the yeast used for challenge, with C57BL/6 and strains bred on that background showing the greatest resistance to lethal challenge. The susceptibility of other strains varies, with mice deficient in complement C5 showing the highest mortality, but no other factors that show a direct correlation with mortality have yet been identified.

C5-deficient mice die rapidly, from acute fungal pyelonephritis. It appears that phagocytic cell recruitment through the chemotactic action of C5 is essential to enable the kidney to overcome the initial burst of fungal proliferation, as passive transfer of C5-sufficient serum led to clearance of the yeast, and survival [1]. Comparison of innate and acquired responses to *C. albicans* in C5-deficient congenic mice demonstrated an earlier and stronger clearance of yeasts from the kidneys in C5-sufficient compared to C5-deficient mice [2], and the effect of C5 deficiency appears to be restricted to the kidney, as other organs and tissues of C5-deficient mice do not

display any unusual susceptibility.

Normal mice challenged intravenously with a sublethal dose of C. *albicans* yeasts develop a systemic infection that closely resembles the human disease, with the brain and the kidney being the organs mostly heavily infected [3]. Although significant deposition of blastospores occurs in other organs, such as lungs, liver and spleen, tissue damage is seldom seen. Histological studies of tissue damage in two inbred strains, BALB/c and CBA/CaH, showed a simple dichotomy in the severity of tissue damage, and other inbred strains conformed to this pattern. Analysis of infection in [BALB/c and CBA/CaH] F1 hybrid mice demonstrated segregation consistent with a simple mendelian co-dominant gene. Using the AKXL recombinant inbred set, this gene (Carg1) was located in the region between 10 and 20cM on chromosome 14, and a second gene *(Carg2)* was also implicated in the expression of tissue damage. The existence of this latter gene was confirmed by analysis of responses in [C57/L x C57BI/6] F1 x C57/L backcross mice, which was again consistent with the behavior of a mendelian co-dominant. The effect of the *Carg2* gene is most evident in mice lacking complement C5, but whether this interaction is synergistic or simply additive remains to be determined. The use of immunodeficient (nude or SCID) mice as models has not been illuminating, as these animals do not show any unusual susceptibility to systemic infection, although the genetically coded differences in tissue susceptibility exhibited by BALB/c and CBA/CaH mice were still evident in nude mice bred on either BALB/c or CBA/ CaH backgrounds [4].

Few other genes have been associated with resistance or susceptibility to C. *albicans*. The *Slc11a1* gene (previously named the natural resistance-associated macrophage protein *1,* Nramp1) located on chromosome 1, controls natural immunity to several infectious agents, such as *Mycobacterium bovis, Leishmania donovani* and *Salmonella typhimurium,* through its pleiotropic effects on macrophage activation and function [5]. In *Candida* infection, macrophage cell lines carrying the resistant allele of this gene acted more effectively against both morphogenic forms of the fungus, and produced more tumor necrosis factor-alpha (TNF-α) in response to stimulation than did congenic cell lines from susceptible mice [6]; however, a role for this gene in host responses *in vivo* remains to be established.

Strain-dependent differences in host responsiveness have also been demonstrated in BALB/cCr and DBA/2Cr mice after infection with the avirulent PCA-2 vaccine strain of C. *albicans* [7]. The outcome of infection correlated with cytokine profiles in T-helper lymphocytes, and comparison of cytokine responses in mouse/yeast strain combinations that induced either self-limiting infections and survival (healer), or chronic disease and death (non-healer), showed that IL-4 and IL-10 production correlated with progressive fatal infection [8]. The relationship of these systemic immune responses to the genetically controlled expression of tissue susceptibility and resistance remains to be explored.

Mucocutaneous candidiasis

Oral infection

Models of oral candidiasis have been difficult to establish, as infection is self-limiting, and clears rapidly in normal mice. DBA/2 mice have been shown to be more prone to infection than BALB/c [9], but any genetic basis for this difference is unknown, although the deficiency of complement C5 in the former strain does not play a role (Farah, unpublished). In contrast to the situation in systemic candidiasis, T cell-deficient nude mice were extremely susceptible to oral challenge, developing a chronic infection that did not clear [10]. The differences in tissue susceptibility between BALB/c and CBA/ CaH mice demonstrated in systemic infection were also apparent after oral challenge, in both normal and nude mice.

Gastrointestinal infection

There have been few studies of mouse strain differences in susceptibility to gastrointestinal infection. BALB/c and DBA/ 2Cr mice that are, respectively, resistant and susceptible to systemic challenge with an avirulent isolate of C. *albicans [7]*, both respond to intragastric inoculation with a virulent yeast by the development of protective responses and eventual clearance of the infection [11]. The implication that systemic and mucosal infections elicit qualitatively different host responses was consistent with the observation that, although germfree SCID mice were susceptible to C. *albicans* infection, and demonstrated chronic candidiasis of the tongue and stomach [12], the infections remained restricted to the mucosa, and no dissemination was observed.

Vaginal infection

The establishment of relevant mouse models of chronic *Candida* vaginitis in women has proven to be an intractable problem. The refractory state of the mouse vagina to colonization with C. *albicans* can be overcome by treatment with oestrogen [13], and mouse strain differences in susceptibility to vaginal infection correlated with estrogen responsiveness of various sub-strains and their F1 hybrids [14]. This model, however, resembles more closely the vaginitis associated with increased hormone concentrations during the third trimester of pregnancy, rather than the recurrent disease. Studies of *Candida* vaginitis in nude mice [15] failed to demonstrate any increase in vaginal yeast burden associated with T cell deficiency. Both nude and control mice had similar levels of vaginal inflammation, but the former demonstrated a more florid fungal growth in the vaginal epithelium.

Candidiasis in gene knockout (KO) mice

In addition to studies on normal and congenitally immunodeficient mice, mice with specific gene deletions have been used to assess and understand the crucial roles of T cells, macrophages and neutrophils, in host defence against mucosal and systemic candidiasis. Three sets of observations are relevant. First, mice lacking the ability to mount T cell-mediated immune responses show no increased susceptibility to systemic candidiasis [16,17], but develop chronic oropharyngeal infection [10]; second, mice that lack the T cell receptor α- and β-chains, and are therefore deficient in α/β and γ/δ T cells, are highly susceptible to oro-gastric candidiasis, but are resistant to acute systemic candidiasis [18]; and third, T cell depletion had no effect on the fungal burden in infected tissues after intravenous infection [19], but increased the severity and duration of oral candidiasis [20]. Overall, these data suggest that there are two discrete pathways of host responsiveness – one demonstrating T cell-dependence and the other in which recovery from infection is predominantly T cell-independent.

Neutrophils are recognized to be a vital early response to C. *albicans* infection, and deletion of genes that affect their recruitment or function typically led to increased susceptibility to infection. TNF-α and lymphotoxin-α (LT) double knockout mice showed a significantly increased mortality following C. *albicans* infection [21], that could be attributed to a dramatic delay in neutrophil recruitment, as well as a reduction in the efficiency of phagocytosis. However, *Candida* killing and production of oxygen radicals by neutrophils from the modified mice was unaffected. The importance of neutrophil recruitment was confirmed by studies in mice lacking the homologue for the IL-8 receptor, which showed increased susceptibility to gastric and acute systemic candidiasis [22], with a slower influx of polymorphonuclear neutrophils into infected tissues, and a reduction in the candidacidal capacity of these cells.

T cells appear to play their role via secretion of cytokines, and it might have been

expected that the use of mice in which specific cytokine genes had been genetically deleted would have provided validation of the pathways proposed to lead to susceptibility and resistance [8]. However, studies of candidiasis in cytokine KO mice has confused, rather than clarified the issue. Interferon-gamma (IFN-γ) is a central mediator of host resistance, but deletion of the IFN-γ gene had significantly different effects in different experimental models, and similar discrepancies were found in the responses of IL-10 and inducible nitric oxide synthase (iNOS) KO mice [23]. By contrast, IL-12 KO mice developed severe oro-pharyngeal candidasis, similar to that in T cell-deficient nude mice that persisted undiminished for at least three months [23]. The function of IL-12 and IFN-γ as mediators of host resistance in this model, and their relative significance, remains to be resolved.

It is generally thought that cytokines such as IFN-γ and IL-12 promote the candidacidal activity of effector cells by stimulating the generation of oxidative agents – a major effector mechanism used by phagocytic cells in killing of *Candida.* As expected, deletion of the gene coding for myeloperoxidase (MPO) or NADPH-oxidase (Phox) resulted in increased susceptibility to pulmonary infections with C. albicans compared with normal mice [24], although Phox KO mice showed a substantially shorter time to death than the MPO-/- mice. In further studies [25], the alimentary tracts of germfree mice deficient for both phagocyte oxidase (Phox) and nitric oxide synthase 2 (NOS2) were colonized with C. *albicans.* All mice quickly became moribund, but this did not reflect a failure of the candidacidal functions of the phagocytic cells, as peritoneal exudate cells from the double KO, and each of the single KO mice, were able to kill C. *albicans in vitro* as efficiently as cells from immunocompetent mice. These experiments suggest that factors other than reactive oxygen or nitrogen intermediates might be sufficient for the killing of phagocytised *Candida* yeasts.

Although a requirement for T cells in the response against oral candidasis has been demonstrated unequivocally, they can function not only by augmenting the candidacidal activity of phagocytic cells but also by increasing their production by the bone marrow [26]. The nature of such an interaction is presently unknown; however, there is increasing evidence for connections between myeloid and lymphoid systems in defense against *Candida.* For example, IL-17A has a regulatory role in inflammation, and mice lacking the receptor for IL-17A showed decreased recruitment of neutrophils to infected tissues [27], with substantially increased colonization of the kidney and reduced survival.

In vitro models

It might be somewhat misleading to refer to *in vitro* systems as 'models' of *Candida* infection, because they are highly artificial, and used mostly in the dissection and analysis of various aspects of the host/yeast interaction. Three important factors that have been amenable to study by these means are adhesion, recognition and effector mechanisms.

The ability of the yeast to adhere to a cell is a fundamental requirement for virulence, and this aspect has been studied extensively using a variety of cell lines, and cells from primary cultures of oral and vaginal epithelium. Adhesion in these models can be influenced by nonspecific factors such as hydrophobicity, as well as by specialized receptor molecules termed adhesins. These include surface glycoproteins such as the iC3b adhesin, the fucose-binding adhesin and the fibronectin adhesin [28], as well as the large family of aspartyl proteinases [29]. Conversely, host cell recognition of *Candida* can occur through several cell surface receptors, of which the Toll-like receptors (TLRs) can be the most important. At present, studies using KO mice have implicated three of these, TLR2, TLR4 and TLR6, in recognition of C. *albicans [30],* but gene profiling of macrophages after interaction with *Candida in vitro* (Li, unpublished) has confirmed that TLR2 is a

major receptor for the yeast. *C. albicans* has the potential to express a variety of pathogen-associated molecular patterns, depending on the growth phase (yeast or filamentous form), that might also influence host responsiveness [31].

Table 1. Comparison of models of *Candida albicans* infection

	In vitro models	*In vivo* models
Pros	1. Analytical 2. Precise 3. Clear answers to specific questions	1. Biologically relevant 2. Can mimic various manifestations of the disease
Cons	1. Artificial 2. Might not address issues involving multiple interactions	1. Complex 2. Can be difficult to interpret 3. Expensive
Best use of model	Dissection of particular pathways or mechanisms	Study of interplay between various effector systems
How to get access to the model	Some useful cell lines are commercially available; organ cultures usually developed by individual investigators	Commercial sources of animals; knockout mice usually obtained from individuals or institutions and bred by the investigator Extensive mouse information is available from the Jackson Laboratory (http://www.informatics.jax.org), and a current list of knockout mice can be found at http://www.immunologylink.com/transgen.htm

Comparison of models

Infectious disease presents a challenge in the development and use of models, as experiments *in vivo* and *in vitro* are generally directed at fundamentally different questions (Table 1). The different manifestations of candidiasis, in particular, have prompted the development of numerous different *in vivo* systems, and hence a reciprocity in the use of *in vivo* versus *in vitro* systems as questions have been answered and others posed, although issues of biological relevance can usually only be resolved definitively *in vivo*.

Conclusion

The complex manifestations of *Candida* infections in humans has led to the development of a variety of animal models of the disease, and it has proven difficult to make valid generalizations from such complex data. However, knowledge of the contribution of various genes to the expression of resistant or susceptible phenotypes will facilitate a more focused approach to the analysis of host responses *in vivo,* and the availability of mice in which genes have been selectively deleted will support this, although the redundancy in cytokine function in cytokine gene knockout mice can make definitive interpretation of such experiments difficult. Many problems and anomalies remain, but with the increasing application of microarray analyses to define patterns of gene activation, both *in vitro* and *in vivo,* a clearer picture of the roles of both innate and adaptive immune responses is gradually emerging.

References

1 Ashman, R.B. *et al.* (2003) Role of complement C5 and T lymphocytes in pathogenesis of disseminated and mucosal candidiasis in susceptible DBA/ 2 mice. *Microb. Pathog. 34,* 103–113

2 Lyon, F.L. *et al.* (1986) Innate and acquired immune responses against *Candida albicans* in congenic B10.D2 mice with deficiency of the C5 complement component. J. *Med. Vet. Mycol.* 24, 359–367

3 Papadimitriou, J.M. and Ashman, R.B. (1986) The pathogenesis of acute systemic candidiasis in a susceptible inbred mouse strain. J. *Pathol.* 150, 257–265

4 Fulurija, *A. et al.* (1997) Increased tissue resistance in the nude mouse against *Candida albicans* without altering strain-dependent differences in susceptibility. J. *Med. Vet. Mycol.* 35, 197–203

5 Kovarova, *H. et al.* (2001) Natural resistance to intracellular pathogens: modulation of macrophage signal transduction related to the expression of the Bcg locus. *Proteomics* 1, 587–596

6 Puliti, *M. et al.* (1995) Influence of the bcg locus on macrophage response to the dimorphic fungus *Candida albicans.* Infect. Immun. 63, 4170–4173
7 Romani, *L. et al.* (1993) Cd4+ subset expression in murine candidiasis. Th responses correlate directly with genetically determined susceptibility or vaccine-induced resistance. J. *Immunol.* 150, 925–931
8 Mencacci, A. et al. (1995) Rationale for cytokine and anti-cytokine therapy of *Candida albicans* infection. J. *Mycol. Med.* 5, 25–30
9 Chakir, J. *et al.* (1994) Differential pattern of infection and immune response during experimental oral candidiasis in BALB/C and DBA/2 (H-2d) mice. *Oral* Microbiol. *Immunol.* 9, 88–94
10 Farah, C.S. *et al.* (2002) Primary role for CD4+ T lymphocytes in recovery from oropharyngeal candidiasis. *Infect.* Immun. 70, 724–731
11 Bistoni, *F. et al.* (1993) Mucosal and systemic T helper cell function after intragastric colonization of adult mice with *Candida albicans. J. Infect. Dis.* 168, 1449–1457
12 Balish, *E. et al.* (1993) Mucosal and disseminated candidiasis in gnotobiotic SCID mice. J. *Med. Vet. Mycol.* 31, 143–154
13 Spanel-Borowski, *K. et al.* (1976) Experimental candida vaginitis in the mouse. *Arch. Gynakol.* 221, 313–322
14 Clemons, K.V. *et al.* (2004) Genetic susceptibility of mice to *Candida albicans* vaginitis correlates with host estrogen sensitivity. Infect. Immun. 72, 4878–4880
15 Black, C.A. *et al.* (1999) Increased severity of *Candida* vaginitis in BALB/c nu/nu mice versus the parent strain is not abrogated by adoptive transfer of T cell enriched lymphocytes. J. *Reprod. Immunol. 45,* 1–18
16 Cutler, J.E. (1976) Acute systemic candidiasis in normal and congenitally thymic-deficient (nude) mice. J. *Reticuloendothel.* Soc. 19, 121–124
17 Rogers, T.J. *et al.* (1976) The role of thymus-dependent cell-mediated immunity in resistance to experimental disseminated candidiasis. J. *Reticuloendothel.* Soc. 20, 291–298
18 Jones-Carson, J. *et al.* (2000) Disparate requirement for T cells in resistance to mucosal and acute systemic candidiasis. *Infect.* Immun. 68, 2363–2365
19 Ashman, R.B. *et al.* (1999) Both CD4+ and CD8+ lymphocytes reduce the severity of tissue lesions in murine systemic candidiasis, and CD4+ cells
also demonstrate strain-specific immunopathological effects. *Microbiology* 145, 1631–1640
20 Farah, C.S. et al. (2001) T cells augment monocyte and neutrophil function in host resistance against oropharyngeal candidiasis. *Infect.* Immun. 69, 6110–6118
21 Netea, M.G. *et al.* (1999) Increased susceptibility of TNF-alpha lymphotoxin-alpha double knockout mice to systemic candidiasis through impaired recruitment of neutrophils and phagocytosis of *Candida albicans. J. Immunol.* 163, 1498–1505
22 Vazquez-Torres, A. et al. (1999) Early resistance of interleukin-10 knockout
mice to acute systemic candidiasis. *Infect.* Immun. 67, 670–674
23 Ashman, R.B. *et al.* (2004) Innate versus adaptive immunity in *Candida albicans* infection. *Immunol. Cell Biol.* 82, 196–204
24 Aratani, *Y. et al.* (2002) Relative contributions of myeloperoxidase and NADPH-oxidase to the early host defense against pulmonary infections with *Candida albicans* and *Aspergillus fumigatus. Med. Mycol.* 40, 557–563
25 Schofield, D.A. *et al.* (2005) Divergent chemokine, cytokine and betadefensin responses to gastric candidiasis in immunocompetent C57BL/6 and BALB/c mice. J. *Med. Microbiol.* 54, 87–92
26 Wanasaengsakul, S. and Ashman, R.B. (2004) Bone marrow colony-formation *in vitro* after infection of genetically defined inbred mice with *Candida albicans. Microb. Pathog.* 36, 211–217
27 Huang, *W.* et al. (2004) Requirement of interleukin-17A for systemic anti-
Candida *albicans* host defense in mice. J. *Infect. Dis.* 190, 624–631
28 Pendrak, M.L. and Klotz, S.A. (1995) Adherence of *Candida albicans* to host
cells. *FEMS Microbiol. Lett.* 129, 103–113
29 Hoyer, L.L. (2001) The ALS gene family of *Candida albicans. Trends Microbiol. 9,* 176–180
30 Roeder, *A. et al.* (2004) Toll-like receptors as key mediators in innate antifungal immunity. *Med. Mycol.* 42, 485–498
31 Gantner, B.N. *et al.* (2005) Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. *EMBO J. 24,* 1277–1286