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Animal models of mucosal *Candida* infection

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Abstract

Rodent models of oral, vaginal and gastrointestinal *Candida* infection are described and discussed in terms of their scientific merits. The common feature of all experimental mucosal *Candida* infections is the need for some level of host immunocompromise or exogenous treatment to ensure quantitatively reproducible disease. A growing literature describes the contributions of such candidiasis models to our understanding of certain aspects of fungal virulence and host response to mucosal *Candida albicans* challenge. Evidence to date shows that T-lymphocyte responses dominate host immune defences to oral and gastrointestinal challenge, while other, highly compartmentalized responses defend vaginal surfaces. By contrast the study of *C. albicans* virulence factors in mucosal infection models has only begun to unravel the complex of attributes required to define the difference between strongly and weakly muco-invasive strains.

Keywords

animal; mucosal; epithelium; oral; model; vaginal

Introduction

Candida species are ubiquitous fungal pathogens and are the most common cause of mucosal and invasive fungal infections in humans. *Candida albicans*, the leading cause of candidiasis, is a polymorphic fungus that reproduces by budding and commonly resides as a commensal in the mucosal tissues of approximately half the human population. When the balance of the normal flora is disrupted or the immune defences are compromised, *Candida* species can become pathogenic, often causing recurrent disease in susceptible individuals. As a result, *Candida* infections are recognized as a serious public health challenge with high medical and social-economical importance. Given the relatively limited number of suitable and effective antifungal drugs, the continuing increase in the incidence of *Candida* infections highlights the need to identify the fundamental pathogenic determinants of *C. albicans* and the reciprocal host protection mechanisms against this fungus at mucosal surfaces (Naglik *et al.*, 2003).

The study of human infectious diseases requires the investigation of microorganisms in model systems. For pathogenic fungi, a number of invertebrate mini-host systems including

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Drosophila melanogaster, *Acanthamoeba*, *Caenorhabditis elegans*, *Dictyostelium discoideum*, and *Galleria mellonella* (Chamilos *et al.*, 2007; Mylonakis *et al.*, 2007) have been developed to study epithelial infections and cellular or humoral responses. Likewise, epithelial organotypic models have been developed for close analysis of the host-fungal interaction at mucosal surfaces (Dongari-Bagtzoglou & Kashleva, 2006; Schaller *et al.*, 2006). These nonvertebrate models offer a number of advantages over mammalian vertebrate models, predominantly due to economy of size and ethical issues (Table 1). However, vertebrate models are invaluable systems for the molecular and cellular analysis of host-fungal interactions as the animals and their environment can be precisely controlled and manipulated to permit thorough investigations of fungal pathogenicity and immunity. Although many of the pioneering studies on mucosal candidiasis were performed in non-human primates (Samaranayake & Samaranayake, 2001), small mammals, including rats and mice, are the common choice for such studies for economical and ethical reasons and because of their relative anatomic and immunological similarity to humans (Samaranayake & Samaranayake, 2001; Chamilos *et al.*, 2007).

A number of clinically relevant rodent models of systemic and mucosal candidiasis have been established, predominantly to study host-pathogen interactions, anti-fungal drug efficacy and pharmacokinetics, and vaccine candidates. Systemic models rely on intravenous injection of *C. albicans*, which is generally an atypical route of infection and will not be covered in this review. Mucosal (oral) inoculation on the other hand more closely mimics the predominant gastrointestinal portal of entry leading to systemic dissemination. However, establishment of mucosal infection models (oral, gastrointestinal, vaginal) generally requires the use of immunosuppressive agents, antibiotic or oestrogen treatment, or the use of germ-free or transgenic animals (Fidel & Sobel, 1999; Samaranayake & Samaranayake, 2001; de Repentigny, 2004). Consequently, when interpreting data from such animal models, one needs to consider the severity of the manipulations before drawing conclusions.

One caveat to studying *C. albicans*–host interactions in rodents is that *C. albicans* is not a natural colonizer of mucosal surfaces in these animals - the rodent equivalent of ‘normal flora yeast’ is *Candida pintolopesii* (Savage &Dubos, 1967), which can sometimes cause infections in immuno-compromised rodents (Kurtzman *et al.*, 2005). This has both benefits and limitations. The advantage is that any host response to *C. albicans* is not affected by pre-existing adaptive immune responses to the fungus. The disadvantage is that, as mentioned above, establishment of mucosal colonization or infection usually requires intervention with immunosuppressive agents, antibiotics or oestrogen. Nevertheless, experimental rodent models have been enormously valuable and our specific emphasis will be on the utility of published models for the study of host-pathogen interactions in superficial *C. albicans* infections.

General aspects of murine and rat mucosal models

The choice of rodent for experimental mucosal *Candida* infection needs to take into account many economic, scientific and ethical considerations (Table 2). In general, the larger the animal host the easier it is to collect repeated samples of fluids and some tissues - a factor which becomes important in experiments designed to monitor, for example, serological parameters over time. However, the higher cost of maintenance and the greater difficulties in handling associated with larger rodent species often favour the use of small rodents, particularly mice. To date, no rodent model of invasive mucosal *Candida* infection has been devised that does not depend on some form of predisposition of the animal by occlusion, immunosuppression, surgical alteration, or elimination of competing microbial flora: often

more than one of these conditions. However, commensal *C. albicans* carriage can be induced in unmodified hosts (Samaranayake & Samaranayake, 2001).

Rodent vaginitis models are highly dependent upon a prolonged state of pseudo-oestrus, which is usually induced and maintained with weekly administration of 17-β-estrodiol (Fidel & Sobel, 1999; Clemons *et al.*, 2004) (Table 2). Additionally, rats must be oophorectomized to obtain a pseudo-oestrus state. Vaginitis models without oophorectomy or the use of oestrogen have been developed, but these are rare and are based on immunosuppression (Tan *et al.*, 2005) or antibiotic treatment (Hamad *et al.*, 2006). Oestrogen levels appear to be the main factor affecting host susceptibility to *C. albicans* and usually a relatively high dose of 100 μg per mouse per week is used to establish infection for 5 weeks or more, although an oestrogen range of 10–500 μg per mouse per week have been reported. In the absence of pseudo-oestrus vaginal infections are usually cleared by the second week (Fidel & Sobel, 1999). Oestrogen transforms the columnar epithelium into thicker stratified squamous epithelium and increases the glycogen content, pH and growth substrates, all of which facilitate *C. albicans* avidity for the tissue and growth (Fidel & Sobel, 1999). Oestrogen may also inhibit innate and/or adaptive immune defences, thus permitting an infection phenotype (Styrt & Sugarman, 1991). In addition, *C. albicans* is known to produce an oestrogen-binding protein (Skowronski & Feldman, 1989) and so addition of mammalian oestrogens might also directly affect fungal colonization or pathogenicity (O'Connor *et al.*, 1998). Because most mouse strains (BALB/c, C57BL/6, DBA/2, CBA/J, C3H/HEN) are susceptible to oestrogen-induced *C. albicans* vaginitis, susceptibility to vaginal candidiasis appears to be independent of the major histocompatibility locus H-2 haplotype (Calderon *et al.*, 2003). However, one exception is the CD-1 mouse, which is innately more resistant to candidal infection, probably due to its resistance to oestrodiol endocrine disruption (Spearow *et al.*, 1999).

Many oral *C. albicans* infection models have been described, with persistent infections usually requiring some form of immunosuppression or intervention therapy (Samaranayake & Samaranayake, 2001)) (Table 2). Historically, the rat was used far more often than the mouse as a host for experimental oral *Candida* infections. Many early studies focused on models in which an acrylic device implanted in the animal's mouth provided the occlusion necessary to establish an infection resembling denture stomatitis. Alternatively, antibacterial treatments predisposed the animals to infection. Without some form of immunosuppression or other manipulation, oral fungal burdens in mice and rats are variable and often decline rapidly. However, it has been demonstrated in both mice (Lacasse *et al.*, 1993) and rats (Jones & Russell, 1973) that after an initial oral infection is established over *c.* 1 week, fungal burdens can reduce naturally to a carrier state for many weeks without the use of immunosuppressive agents. To establish oral candidiasis in mice, animals are usually treated with steroids (Deslauriers *et al.*, 1995), often with the addition of antibiotics such as tetracycline (Kamai *et al.*, 2001). Although fungal burdens can be initially high, burdens diminish to very low levels after cessation of drug treatment (Deslauriers *et al.*, 1995).

One model that has been lacking, but which recently has been developed, is a model of concurrent oral and vaginal colonization in the same mouse, which has the added ethical benefit of reducing the required numbers of experimental mice by half (Rahman *et al.*, 2007). This model appears successful due to the use of a fresh clinical oral strain of *C. albicans* (529L) and the administration of low doses of oestrogen (5 μg) via both intramuscular and subcutaneous routes. Colonization with *C. albicans* 529L was shown to be relatively high over a 5–6 week period and similar to that generally found during human mucosal colonization. Manipulation of this model to induce an infection state would be highly desirable, and would permit the detailed investigation of host-*Candida* interactions that exist during both the colonization and infection states at multiple sites.

Gastrointestinal candidiasis in humans often arises in patients treated with high dose immunosuppressants or cancer chemotherapy (Pappas *et al.*, 2004). To mimic this condition, a number of gastrointestinal models have been developed in the mouse, many of which have been used to test the efficacy of antifungal drugs (Clemons *et al.*, 2006; Capilla *et al.*, 2007). Gastrointestinal infection models in the rat have occasionally been described, including models in which some animals developed measurable visceral burdens (Wong *et al.*, 1990). The advantage of the larger rodent model in permitting repeated blood sampling for diagnostic purposes was well demonstrated in these studies. Establishing a model of disseminated candidiasis that arises from translocation of *C. albicans* across the gastrointestinal mucosa has been more challenging, but has been created in infant mice (aged 4–6 days), in which dissemination of *C. albicans* to the liver kidney and spleen could be detected up to 72 h after intra-gastric inoculation (Pope *et al.*, 1979). However, the inconsistent visceral dissemination and low visceral fungal burdens arising in this model are typical of attempts to devise gut translocation systems in a variety of hosts. A mouse model recently developed using a specific regime of antibiotics and 5-fluorouracil appears more promising (Clemons *et al.*, 2006), and seems useful not only for drug efficacy studies but also for fungal pathogenicity studies. Further developments in this area are necessary to advance our understanding of host-pathogen interactions at the gastrointestinal mucosa.

Germ-free or gnotobiotic mice appear uniquely suited for studies of mucosal candidiasis, because mucosal surfaces can be naturally and chronically colonized by *C. albicans* without the need for trauma, immunosuppressive or antimicrobial therapy. In addition, colonization can persist for the lifetime of the animal (Balish *et al.*, 1990; Rahman & Challacombe, 1995). However, the germ-free/gnotobiotic model is not ideal for the study of fungal dissemination from mucosal sites or natural host-pathogen interactions, because the absence of the normal microbiota is likely to have a major effect on both fungal pathogenicity and the host response.

The utilization of transgenic or congenitally immunodeficient mice has been instrumental in advancing our understanding of the critical roles of $CD4^+$ (helper) T cells, $CD8^+$ (cytotoxic) T cells, γδT cells, polymorphonuclear leukocytes (PMNs), macrophages, and cytokines in host defence against mucosal candidiasis. These data indicate that protection against mucosal candidiasis involves the cooperation of several immune cell populations and molecules, which together can prevent invasion of mucosal surfaces by *C. albicans* in the normal host. It is thus evident that multiple, rather than single, defects in host defence mechanisms potentially underlie mucosal candidiasis.

Study of *Candida* pathogenesis in mucosal animal models

In humans, isolation of *Candida* species from mucosal tissues does not indicate a diseased state and over the past decade it has become clear that it is the physiological status of the host that is the primary factor governing the aetiology of candidiasis. However, it appears that the transition from harmless commensal to unrelenting pathogen is a fine line and one that is attributable to an extensive repertoire of virulence determinants selectively expressed under suitable predisposing conditions (Naglik *et al.*, 2003). Animal models have been used to determine the importance and relative contribution of *C. albicans* pathogenic attributes to *in vivo* infections through a variety of experimental approaches, including gene expression (reverse-transcription PCR and *in vivo* expression technology), protein inhibition, and use of *C. albicans* mutant strains (gene disruption/knockouts) (Naglik *et al.*, 2003; de Repentigny, 2004; Brown *et al.*, 2007; Hoyer *et al.*, 2007).

The majority of these studies have been performed using either *in vitro* models or systemic intravenous models of infection; significantly fewer have been undertaken in animal models

of mucosal infection. This is likely due to the relative difficulty in establishing and maintaining mucosal models of infection in comparison with systemic models and underscores the absence of a clear, universally reproducible mucosal model to parallel the widely used disseminated model based on intravenous challenge. One of the most common and best-accepted approaches for identifying virulence genes in *C. albicans* is to create gene-disrupted mutant strains and to assess them *in vivo.* A large number of genes have been disrupted in *C. albicans* but only a minority have been investigated in mucosal infection models (Table 3): most of the genes listed are better known as putative virulence factors in systemic infection models. Therefore, many of the factors that have been concluded to be pathogenic determinants during systemic infections have yet to be confirmed as pathogenic determinants during mucosal infections. For example, we have previously demonstrated that several clinical mucosal isolates were less virulent than laboratory isolates in a systemic model but similarly virulent in a vaginal model of infection model (Taylor *et al.*, 2000a). As such, our current understanding of mucosal *Candida* infections *in vivo* remains relatively elementary. More detailed investigations using animal mucosal models will be essential to confirm and to identify novel virulence factors during epithelial infections.

One of the reasons why virulence studies in animal mucosal models are sparse is because of the use of *C. albicans* strain SC5314. Although almost universally utilized in systemic pathogenicity studies and other molecular-based investigations, SC5314 has limited use in mucosal pathogenicity studies because it is a very poor colonizer of epithelial surfaces *in vivo*, and particularly of mice (Taylor *et al.*, 2000a; Rahman *et al.*, 2007). Given that nearly all current *C. albicans* mutants are created in the CAF2-1, CAI4, BWP17 background, all of which derive from the wild type strain SC5314, potential errors are likely to be made in concluding a role (or lack of a role) for specific genes during mucosal *C. albicans* infections. To determine the role of putative virulence genes in mucosal infections more comprehensively, gene-disruption mutants would need to be created (or recreated) in a number of clinical wild-type strains known to colonize or infect mucosal surfaces, such as *C. albicans* strain 529L (Rahman *et al.*, 2007). Utilizing these ‘mucosal’ *C. albicans* strains provides plenty of scope to identify novel genes, proteins, and pathways that might be specifically or preferentially required to colonize and infect epithelial cells *in vivo*. This is particularly relevant to oral and vaginal infections where stresses and environmental signals (e.g. temperature, pH, alternative nutrients, osmotic stress, oxygen concentration) are quite different to each other, let alone from those found during systemic infections.

Apart from the use of SC5314, other problems in mucosal animal experimentation are that the read-out is often based solely on fungal burdens. Very few studies include histological analysis, which would allow anatomical and morphological interactions to be assessed more closely (for example, a virulent strain may cause damage without multiplying extensively in the organ), or analysis of the host response, which would determine whether deficiencies in these putative virulence genes have a direct effect on how the host recognizes or responds to the fungus. This is a common problem of many studies in general which often target the fungus or the host, rather than investigating the two together. Other shortcomings include the effect of *Candida* preculture growth, the route of inoculation, innate differences between *C. albicans* strains, and the need to include both the disrupted mutant and the reintegrant strains, which is often lacking in animal pathogenicity studies (Table 3). Together, these data emphasize the need to develop current models and to establish more sophisticated models of *C. albicans* colonization and infection in order to advance our understanding of *C. albicans* pathogenicity at mucosal surfaces.

Study of *Candida* immunity in mucosal animal models

Host defence against *C. albicans* and most microorganisms fall into three categories: nonspecific (mucosal barrier, commensal bacteria, natural secretions), innate immunity based on PMN leukocytes and macrophages, and adaptive immune mechanisms based on T- and B-lymphocytes. Although mucosal animal models have not been utilized to their potential to study *Candida* pathogenesis, their use has been instrumental in deciphering the roles of specific immune components against *Candida* infections (Table 4). Animal studies have taught us that host defence against mucosal infections involves several arms of the immune response, that protective mechanisms differ in the vagina compared with oral/gastrointestinal, and that vaginal immune responses are compartmentalized and highly regulated.

Strong evidence exists indicating that cellular immunity, mediated by $CD4^+$ Th1 cells, protects against oral and gastrointestinal *C. albicans* infection. This was demonstrated experimentally in animals using congenitally immunodeficient, transgenic mice and virus-infected mouse models (Table 4) and clinically in humans, in which various immunocompromised population groups (e.g. AIDS, transplant and steroid therapy) are more susceptible to oropharyngeal candidiasis (Klein *et al.*, 1984). Additionally, $CD4^+$ T cell protection appears to work in combination with certain cytokines, including IL-12, IFN-γ, IL-4, TNF-α, and nitric oxide (Elahi *et al.*, 2000, 2001; Farah *et al.*, 2002a, 2002b). During murine vaginitis, a Th1-type *Candida*-specific systemic cell mediated immune (CMI) response can also be generated. However, unlike in the oral mucosa, the systemic CMI response does not appear to be the predominant protective defence mechanism against *C. albicans* vaginal infections, as no correlation was observed between vaginal T-helper, proinflammatory cytokines and fungal burden (Fidel, 2007). Rather, local mucosal immunity distinct from that in the peripheral circulation, together with immunoregulation by γδ T cells and dendritic cells (DCs), is now under consideration as the main immune mechanism governing at the vaginal mucosa (Fidel, 2007) (Table 4). The role of $CD8^+$ T cells in protection against mucosal infection is more contentious than for $CD4^+$ cells. In rats, $CD8^+$ cells are more numerous than $CD4^+$ cells in the vagina (similar to humans), whereas in mice $CD4^+$ T cells are exclusive (Cassone *et al.*, 2007). Irrespective, the current data indicate that $CD8^+$ T cells may play protective roles orally and gastrointestinally, but not vaginally (Table 4).

Importantly, T cells alone do not appear to protect against systemic candidiasis of mucosal origin because neither ν/ν (T cell deficient) nor ν/+ (T cell competent) mice develop progressive systemic disease (Balish *et al.*, 1990). Protection against systemic candidiasis of mucosal (oral/gastrointestinal) origin is predominantly mediated by phagocytic cells (PMNs and macrophages) (Cantorna & Balish, 1990). This is supported by a recent study which demonstrated protection from gastrointestinal transmission of *C. albicans* across the mucosa by bombesin (Algin *et al.*, 2005), a 14 amino acid peptide from frogs that stimulates all steps of the phagocytic process in murine peritoneal macrophages. Interestingly, PMN do not play an obvious protective role in the vaginal lumen (Table 4), and indeed in humans PMNs appear to exacerbate the vaginal disease (Fidel *et al.*, 2004b). The reason for this is as yet unknown. Nonetheless, animal data strongly indicate a fundamental role for PMNs in protection against systemic candidiasis of mucosal (oral/gastrointestinal) origin.

T regulatory (Treg) and Th17 cells (T cells that secrete IL-17) have recently emerged as important immune cells controlling immune responses to *Candida* infections (Netea *et al.*, 2004; Yu & Gaffen, 2008). There is evidence for the presence of $CD25^+$ $CD4^+$ Treg cells in the vagina (Wormley *et al.*, 2001) and the Th17 pathway is activated in response to disseminated *C. albicans*, which is thought to negatively regulate the Th1-mediated

resistance pathway (Zelante *et al.*, 2007; Huang *et al.*, 2004). However, the precise functional roles of Treg and Th17 cells during mucosal fungal disease remain unclear. This is one area in which animal mucosal models can be specifically utilised to answer crucial questions concerning the roles of these two T cell types against superficial *C. albicans* infections.

Other immune cell types include DC's, which represent the crucial link between innate and adaptive immunity and orchestrate cell mediated and humoral defence mechanisms. DC's contribute to protection or immunoregulation against *C. albicans* infection in the gastrointestinal or vagina, respectively, but their protective role in oral tissues is undefined (Table 4) (De Bernardis *et al.*, 2006). Conversely, natural killer cells are not thought to convey strong protection at any mucosal site (Table 4). Currently, there also is a lack of evidence concerning the protective role of naturally induced antibodies against mucosal *Candida* infection. However, although antibody levels against *C. albicans* appear unaltered irrespective of the disease state, there is good evidence from immunization studies that a limited subset of antibodies are able to protect against mucosal *Candida* infection (Table 4). This suggests that animal models have been useful in identifying immunotherapeutic and neutralizing antibodies, which could potentially be generated for clinical use. However, a more detailed analysis of the functional characteristics of these protective antibodies and their target epitopes is required.

More recently, epithelial cells and cytokines have emerged as potentially protecting and/or modulating the local immune response during *Candida* infections (Dongari-Bagtzoglou & Fidel, 2005; Fidel, 2007). Epithelial cells secrete high levels of the immunoregulatory cytokine TGF-β that promotes antibody class switching to IgA (the protective mucosal antibody) (Taylor *et al.*, 2000b), numerous proinflammatory cytokines (Schaller *et al.*, 2002; Dongari-Bagtzoglou & Fidel Jr., 2005) and antifungal peptides (Cole, 2006; Kazmi *et al.*, 2006). Indeed, vaginal epithelial cells from mice and non-human primates are able to inhibit *Candida* growth *in vitro* (Table 4), and oral epithelial cells collected from saliva of humans have similar activity (Fidel, 2006). However, an oral epithelial-mediated inhibitory effect has not yet been demonstrated in animal models. In addition, in an *in vitro* model, human epithelial cells can also protect the oral mucosa from *C. albicans* infection directly through epithelial Toll-like receptor 4 (TLR4) via a process mediated by PMNs and TNFα (Schaller *et al.*, 2004; Weindl *et al.*, 2007). The protective role of TNFα at mucosal surfaces supports other clinical human data (Moen *et al.*, 2005) but also animal data, in which oral administration of recombinant TNFα reduces fungal burdens (Ohta *et al.*, 2007) and where TNFα knockout mice are more susceptible to oral *C. albicans* infection (Farah *et al.*, 2006). The role of cytokines/chemokines in the protection against vaginal candidiasis is uncertain, but monocyte chemotactic protein-1(MCP-1) appears to play some role in reducing the fungal burden during vaginal infection as intravaginal neutralization of MCP-1 with antibodies results in a significant increase in vaginal fungal burden during infection (Saavedra *et al.*, 1999). Given that the mucosal epithelium is the primary cell layer that initially encounters *Candida*, it is paramount that animal models are utilised to investigate the importance of epithelial cells and their associated innate factors in host defence.

Summary comments and future directions

Experimental animal models of mucosal candidiasis have been invaluable in assessing fungal pathogenicity and host immune defences. These studies have informed us that *C. albicans* is the most virulent *Candida* species *in vivo* and that the immune response to *Candida* is different at the various mucosal sites and is compartmentalized and highly immunoregulatory in the vagina. These findings, in turn, have highlighted the gaps in our knowledge of *Candida* pathogenicity and host immunity and provide clues as to how they

might be addressed. To more accurately determine the role of the host in the host-pathogen interaction, a model is required in which specific host proteins or processes can be manipulated in order to permit either colonization or disease in the same animal. Likewise, to obtain a more global picture of why the immune response is distinct at different mucosal sites, various arms of the immune response (innate, CMI, humoral) need to be investigated at different mucosal sites (oral, vaginal and gastrointestinal) within the same animal strain. All too often only a single arm of the immune response is assessed and a single model utilized.

Specific areas for future research include the identification of surface recognition receptors on epithelial cells that bind *Candida* and initiate the innate immune response. Currently, only E-cadherin has been identified as an epithelial receptor for *C. albicans* (via Als3) permitting mucosal invasion (Phan *et al.*, 2007). Although a number of receptors are known to recognize *C. albicans* on other immune cells [TLRs, dectin-1, dendritic cell-specific intercellular adhesion molecule 3 grabbing nonintegrin (DC-SIGN), mannose receptor, complement receptor 3, galectin-3], these have not yet been confirmed as genuine receptors on epithelial cells that can initiate immune responses. Experimental mucosal models using animals deficient in these receptors are necessary to determine their precise role in fungal recognition at epithelial surfaces. In addition, the signalling pathways in epithelial cells that orchestrate the subsequent immune response are undefined. Deciphering these pathways should provide vital clues as to how *C. albicans* is detected and whether the fungus manipulates epithelial signalling systems for its own benefit (e.g. to permit colonization). Moreover, little is known about the identity of the inhibitory proteins that prevent signalling activation in epithelial cells. This might provide an explanation for the considerable amount of immunoregulation that is observed at mucosal surfaces, and specifically in the vagina. Other areas that need to be explored are the precise roles of the Th1/Th2/Th17/Treg pathways in defence against mucosal diseases, and the interaction between epithelial cells and other cells of the immune system. This will be greatly facilitated by the use of transgenic or knockout mice, together with targeted investigation of the fungal cell wall and fundamental biological processes such as hypha formation.

Over the past two decades we have come far in our understanding of host-*Candida* interactions at mucosal surfaces. Over the next decade the fungal community can take advantage of advances in molecular, cellular, immunological and genomic/proteomic technologies, as well as mutant strain libraries and systems biology approaches in relevant animal models to more fully decipher the complex interactions that take place between this eukaryotic ‘opportunistic commensal pathogen’ and its mammalian host.

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Table 1

Benefits and limitations of vertebrate and nonvertebrate mini-host models in studying the pathogenesis of *Candida* infections

Model system	Benefits	Limitations
Nonvertebrate mini-host	Host and environment can be precisely controlled Reproducibility Low cost, simplicity, rapid results High throughput preliminary screening for strain virulence and responses to antifungal agents Well characterized genetics and immunity that can be manipulated to study host–*Candida* interactions Many innate immune mechanisms conserved between invertebrates and mammals, permitting limited investigation of innate responses to *Candida* Fewer ethical concerns	Precise inocula can be difficult Molecular and cellular host mechanisms may not be evolutionarily conserved with mammalian species Absence of most mammalian compartments and immune cells makes models over-simplistic (e.g. lack of adaptive immunity) Mammalian specific pathogens and virulence determinants can not be investigated/identified Do not provide a good substrate for modelling superficial infections Effects of antifungal agents similar to *in vitro* tests Many model hosts require experimentation at low temperatures (<30 °C)
Vertebrate (mouse/rat)	Host and environment can be precisely controlled Evolutionarily closer to humans, with anatomical and immunological similarity: host–*Candida* interactions more relevant to humans Allow development of superficial and disseminated infection models Amenable to genetic studies: knock-out and knock-in gene studies allow investigation of gene (product) contribution to the *whole* infectious process Precise inocula via specific routes of inoculation	Large scale studies are time-consuming and prohibitive on cost Severe ethical constraints Physiologic and anatomic complexity Quantitative outcome reproducibility a problem: usually overcome by using conditions and challenge doses that are not representative of natural human infections Potentially difficult antifungal pharmacokinetics in some species mean responses may not be extrapolated to effects in humans Long reproductive cycles

Table 2

Comparison of rat vs. mouse mucosal models in studying host–*Candida* interactions

	Mouse		**Rat**	
	Advantage	**Disadvantage**	**Advantage**	**Disadvantage**
General	Cost Species-specific reagents No adaptive immune response to *Candida* in naïve state Availability and ease of production of genetically modified strains	Not colonized with *C. albicans* like humans	No adaptive immune response to *Candida* in naïve state	Not colonized with *C. albicans* like humans Lack of some species-specific reagents
Vaginal model	Not mouse strain dependent Vaginal lavage to quantify fungal burden No vaginal CD8 T cells Efficacy/predictability of drug studies Parallel to human infection	Requirement for pseudoestrus Too robust with estrogen; not robust enough without pH (neutral) Lack of symptoms Short half-life of drugs Repeat vaginal lavage during longitudinal studies	Spontaneous clearance of infection. Longitudinal evaluation Vaginal CD8 T cells Efficacy/predictability of drug studies	Requirement for pseudoestrus pH (neutral) Lack of symptoms Oophorectomy to establish pseudoestrus
Oral model	Ease of inoculation (cotton swab)	Need for immunosuppression to establish persistent infection Poor for vaccine efficacy studies	Ease of inoculation (cotton swab)	Need for immunosuppression to establish persistent infection Poor for vaccine efficacy studies
GI model	Fecal specimen quantification of fungal burden	Lack of persistent infection as detected by fecal specimen	Not characterized	Not characterized

Table 3

Animal mucosal models used to study *Candida* pathogenicity in 'molecular Koch's postulates' experimental designs

Animal model	Gene	Biological function	Result of gene disruption	Reintegrant tested?	References
Mouse oral	*ALS1*	Surface glycoprotein	Lower counts early in infection	Y	Kamai *et al.* (2002)
Mouse oral	*CKA1/2*	Protein kinase	Reduced virulence	Y	Chiang et al. (2007)
Mouse oral/gastric	*PLB1*	Phospholipase B	Reduced ability to cross gut wall	Y	Mukherjee *et al.* (2001)
Mouse oral/gastric	*PLD1*	Phospholipase D	Reduced virulence	N	Dolan *et al.* (2004)
Mouse gastrointestinal	*GPI7*	Involved in GPI anchor attachment	Reduced colonization	N	Richard *et al.* (2002)
Mouse gastrointestinal	*EFH1*	Transcription factor involved in hypha formation	Increased colonization	Y	White *et al.* (2007)
Rat oral	*ADE2*	Nucleotide synthesis	Reduced oral colonization	N	Cole *et al.* (1995)
Rat oral	*FAS2*	Lipid biosynthesis	Reduced oral colonization	Y	Zhao *et al.* (1996)
Rat oral	*URA3*	Nucleotide synthesis	Reduced oral colonization	N	Cole *et al.* (1995)
Piglet oral	*EFG1*	Transcription factor involved in hypha formation	Reduced virulence	Y	Riggle *et al.* (1999)
Mouse vaginal	*CSH1*	Surface hydrophobicity	Reduced colonization	Y	Singleton *et al.* (2005)
Mouse vaginal	*NOT5*	Required for hypha formation	No effect	Y	Cheng *et al.* (2005)
Rat vaginal	*HK1*	Two-component histidine kinase	No effect	Y	Calera *et al.* (1999)
Rat vaginal	*MNT1*	Mannosyl transferase	Reduced virulence	N	Buurman *et al.* (1998)
Rat vaginal	*PHR1*	pH-regulated gene	No effect	Y	DeBernardis *et al.* (1998)
Rat vaginal	*PHR2*	pH-regulated gene	Essential for virulence	Y	DeBernardis *et al.* (1998)
Rat vaginal	*SAP1*	Secreted proteinase	Reduced virulence	N	DeBernardis *et al.* (1999)
Rat vaginal	*SAP2*	Secreted proteinase	Reduced virulence	Y	DeBernardis *et al.* (1999)
Rat vaginal	*SAP3*	Secreted proteinase	Reduced virulence	N	DeBernardis *et al.* (1999)
Rat vaginal	*SAP4-6*	Secreted proteinase	No effect	N	DeBernardis *et al.* (1999)

Table 4

Host defences against experimental mucosal *Candida* infections

	Mucosal site (model)			
Host defence mechanism Innate	**GI**	**Oral**	**Vaginal**	**Reference (reviews)**
PMNs	+/− (ms[*])	+(ms)	− −/++[†] (ms)	Fidel & Finkel-Jimenez (2006); Fidel (2007)
Macrophage	+/− (ms)	+(ms/rat)	− − (ms)	Fidel & Finkel-Jimenez (2006); Fidel (2007)
Natural killer cells	− − (ms)[‡]	? (ms)	− − (ms)	Fidel & Finkel-Jimenez (2006); Fidel (2007); Westwater et al. (2007)
Dendritic cells	++	?	++[§] ++[¶] (rat)	Fidel & Finkel-Jimenez (2006); Fidel (2007)
Epithelial cells	?	?	++ (ms/monkey)	Fidel & Finkel-Jimenez (2006); Fidel (2007)
Humoral				
IgM	− − (ms)	?	+ (ms)	Fidel & Finkel-Jimenez (2006); Fidel (2007)
IgG	− − (ms)	?	+/− (rat/ms)	Fidel & Finkel-Jimenez (2006); Fidel (2007)
IgA	− − (ms)	?	+ (rat/ms)	Fidel & Finkel-Jimenez (2006); Fidel (2007)
Cell-mediated				
$CD4^+$ T cells (Th1)	++ (ms)	+++(ms)	+/− (rat/ms)	Fidel & Finkel-Jimenez (2006); Fidel (2007)
$CD8^+$ T cells[//] (cytotoxic)	+(ms)	++ (ms)	− − (rat/ms)	Fidel & Finkel-Jimenez (2006); Fidel (2007)
Treg/Th17	?	?	+ (Treg)	Fidel & Finkel-Jimenez (2006); Fidel (2007)

− −, fully negative; +/−, evidence for and against in the respective model in brackets (exception is [†]); +, ++, +++, different degrees of positive involvement; ?, unknown involvement at this stage.

[*]Ms, mouse.

[†]Activity is associated with susceptibility to infection rather than protection (unpublished, Fidel) parallel to the clinical condition (Fidel *et al.*, 2004a).

[‡]Not based on the models, but from conventional research efforts.

[§]Plamacytoid dendritic cells involved in immunoregulation.

[¶]Shown to be protective.

[//]May be innate response rather than adaptive.