Virulence 5:2, 240–242; February 15, 2014; © 2014 Landes Bioscience

In vitro models of hematogenously disseminated candidiasis

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Keywords: virulence, endothelial cells, epithelial cells, adherence, invasion, damage

In vitro models, in which *Candida albicans* is incubated with one or more type of host cell, are a powerful tool for dissecting the pathogenesis of infection. Using these models, investigators have elucidated important responses of both the host and the fungus that influence the outcome of infection. In addition, tissue culture models have been invaluable for discovering host cell receptors for *C. albicans*, their cognate fungal ligands, and downstream signaling pathways. Furthermore, in vitro models can serve as surrogates for in vivo infection, facilitating the discovery of key host defense mechanisms and fungal virulence factors. Although tissue culture models of infection have numerous strengths, they also have weaknesses that preclude them from completely replacing animal models of infection.

C. albicans is an opportunistic pathogen that typically requires dysfunction of the host innate immune system to cause invasive infection. Because the interactions of *C. albicans* with the innate immune system are a key factor in determining the outcome of infection, the interactions of *C. albicans* with professional phagocytes has been modeled using macrophage cell lines. For example, Lorenz and Fink used the J774 mouse macrophage cell line to discover that the glyoxylate cycle plays a key role in *C. albicans* virulence.[1] Macrophage cell lines have also been invaluable for identifying the receptors that enable phagocytes to recognize specific components of the *C. albicans* cell wall. Receptors for *C. albicans* that have been discovered using macrophage cell lines include dectin-1,[2] dectin-2,[3] mincle,[4] galectin-3,[5] the macrophage mannose receptor,[6] and integrin $\alpha_M\beta_2$.[7] Notably, the importance of these receptors for the host defense against disseminated candidiasis in vivo has been confirmed using mutant strains of mice that lack these receptors. Furthermore, studies of humans with a naturally occurring stop mutation in the *DECTIN-1* gene demonstrate that dectin-1 is necessary for the host defense against mucosal candidiasis.[8] This strong correlation between in vitro and in vivo results supports the utility of the macrophage model for studying the interactions between *C. albicans* and professional phagocytes.

A key issue with macrophage studies is the source of the cells. The mouse J774 and RAW 264.7 macrophage cell lines are particularly useful for in vitro studies because they are well-characterized, numerous mutant strains are available, and they are relatively easy to transform. However, a limitation of these cell lines is that they are very poor at killing *C. albicans*. This limitation can be overcome by using primary mouse macrophages, either derived from bone marrow cells or elicited from the peritoneum. Because these macrophages are from mice, they are highly likely to be predictive of the interactions of *C. albicans* with phagocytes during invasive infection in these animals. Nevertheless, mouse macrophages differ significantly from their human counterparts.[9] For example, mouse macrophages produce high amounts of nitric oxide (NO), which contributes to microbial killing, whereas human macrophages produce much lower levels of NO and kill microorganisms by NO-independent mechanisms.[10] Because of the differences between mouse and human macrophages, it is important to use human macrophages, preferably primary cells, to verify results obtained with mouse macrophages.

While growing on mucosal surfaces and during invasive infections, *C. albicans* interacts not only with professional phagocytes, but also with other types of host cells, including endothelial and epithelial cells. In vitro endothelial cell models have been used to dissect the pathogenesis of hematogenously disseminated candidiasis because, during the initiation of this disease, *C. albicans* must adhere to and invade the endothelial cell lining of the blood vessels to infect the deep tissues.[11] The majority of studies have investigated the interactions of *C. albicans* with human umbilical vein endothelial cells under static conditions. Studies with this model indicate that multiple members of the *C. albicans ALS* gene family mediate endothelial cell adherence.[12-18] Also, *C. albicans* invades these cells by induced endocytosis, which is triggered when Als3 and Ssa1 on the surface of hyphae bind to N-cadherin and other receptors on the endothelial cell surface.[19-22] In addition, *C. albicans* invasion of endothelial cells is associated with the induction of endothelial cell damage and stimulation of a pro-inflammatory response.[23-28]

A limited number of studies examined the interactions of *C. albicans* with endothelial cells under conditions of flow. One such study determined that *C. albicans* hydrophobic proteins mediate endothelial cell adherence.[29] Two different groups have investigated the relative adherence of hyphae vs. yeast–phase organisms under conditions of flow. Grubb et al.[30] found that

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Submitted: 12/04/2013; Accepted: 12/06/2013
http://dx.doi.org/10.4161/viru.27453
Comment on: Szabo EK, Maccallum DM. A novel renal epithelial cell in vitro assay to assess *Candida albicans* virulence. Virulence 2014; 5:286-96; PMID:24225657; http:/dx.doi.org/10.4161/viru.27046

C. albicans yeast were more adherent than hyphae to endothelial cells under flow conditions. In contrast, Wilson and Hube[31] determined that hyphae were more adherent than yeast under these conditions. Although flow assays may mimic the interactions of *C. albicans* with endothelial cells in vivo more accurately than static assays, to date no new *C. albicans* virulence factor has been discovered using flow assays.

C. albicans clearly interacts differently with endothelial cells from different vascular beds. For example, brain microvascular endothelial cells uniquely express the gp96 heat shock protein on their surface.[32] A *C. albicans vps51*Δ/Δ mutant has increased surface exposed Als3 and increased binding to gp96. Although this mutant has enhanced invasion of human brain microvascular endothelial cells, it has impaired invasion of human umbilical vein endothelial cells. As predicted by these in vitro results, the *vps51*Δ/Δ mutant has increased trafficking to the brain, but reduced trafficking to the kidneys in the mouse model of disseminated candidiasis.[33] Collectively, these results suggest that *C. albicans* utilizes different endothelial cell receptors to invade different vascular beds. Another type of endothelial cell that has been used for in vitro studies is the HMEC-1 cell line, which was developed by transfecting the simian virus 40A gene into human foreskin dermal microvascular endothelial cells.[34] In comparison studies, *C. albicans* adheres to, invades, and damages human umbilical vein endothelial cells more than HMEC-1 cells. Also, *C. albicans* infection stimulates human umbilical vein endothelial cells, but not HMEC-1 cells to secrete the chemokine, interleukin-8.[35] As discussed below, the capacity of *C. albicans* mutants to invade and damage human umbilical vein endothelial cells is a relatively good predictor of their virulence in the mouse model of disseminated infection. Whether the interactions of *C. albicans* with HMEC-1 cells also correlate with virulence is not yet known.

In general, the capacity of *C. albicans* mutants to damage normally non-phagocytic cells in vitro is a fairly good indicator of their virulence in the mouse models of infection. The correlation between host cell damage and virulence has been demonstrated most clearly using the human umbilical vein endothelial cell model.[19,36-38] However, some mutant strains have significantly impaired capacity to damage endothelial cells in vitro, yet have normal or even increased virulence during disseminated infection in mice. These strains include the *als3*Δ/Δ, *tpk2*Δ/Δ, and *pra1*Δ/Δ mutants.[20,39-43] One explanation for these results is that *C. albicans* is exposed to different conditions in the mouse compared with cultured host cells in vitro. Thus, unique signaling pathways may be activated in vivo that compensate for the absence of the gene of interest. Indeed, Fanning et al.[44] found that the Bcr1 transcription factor has different downstream targets in vivo compared with in vitro. Another explanation is that the interactions of *C. albicans* with professional phagocytes are as important as its interactions with normally non-phagocytic cells in determining the outcome of infection. For example, although the *pra1*Δ/Δ mutant is defective in damaging endothelial cells in vitro, it is resistant to neutrophil killing, and this resistance is the likely cause of the increased virulence of this strain.[7,42,43] Furthermore, some strains of *C. albicans*, such as the *sun41*Δ/Δ mutant, do not have defects in damaging endothelial cells, but have attenuated virulence in mice.[45] In this case, the cell wall integrity defects of the mutant likely render it susceptible to phagocyte killing, even though these defects do not influence its interactions with endothelial cells.

In the current issue, Szabo and MacCallum describe the interactions of *C. albicans* with the M-1 mouse kidney cortical epithelial cell line, which was established from a transgenic mouse that expressed the early region of simian virus 40.[46,47] They show that *C. albicans* invades and damages these cells and induces them to secrete the chemokines KC and MIP-2. In addition, multiple clinical isolates and mutant strains of *C. albicans* with known virulence defects in the mouse model of hematogenously disseminated infection cause less damage to these renal epithelial cells and induce lower chemokine secretion. Based on these data, the authors propose that this in vitro model can be used to assess the virulence potential of *C. albicans* strains. While the authors' data are compelling, it would be interesting to know how this cell line compares with the endothelial cell model for predicting the virulence of various *C. albicans* strains, especially those for which in vitro results correlate poorly with in vivo virulence.

Nevertheless, because *C. albicans* is exposed to such a wide range of host cells and diverse microenvironments during a disseminated infection, it is extremely unlikely that any in vitro model can predict the virulence of all *C. albicans* strains. Thus, even though in vitro models of infection are extremely useful for investigating host–pathogen interactions, experimental animal models of infection are still required to verify in vitro results.

Disclosure of Potential Conflicts of Interest

S.G.F. is a co-founder and shareholder of NovaDigm Therapeutics, Inc.

Acknowledgments

S.G.F. is supported by grant R01AI054928 from the National Institutes of Health USA.

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