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Galleria mellonella as a model host to study virulence of *Candida*

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Candida spp. are among the most important human fungal pathogens, with infections ranging from relatively benign, superficial manifestation to life-threatening deep-seated candidiasis and disseminated disease. Different mouse models have been developed to recapitulate the different forms of candidiasis and murine models are considered to be the gold standard to study pathogenesis and analyze efficacy of antifungal treatment. However, economical, logistical, and ethical considerations limit the use of mammals in infection experiments, especially when the question at hand requires analysis of a large number of fungal strains. As an alternative approach, different invertebrate infection models have been developed, including *Caenorhabditis elegans*, *Drosophila melanogaster*, and larvae of the wax moth *Galleria mellonella* as hosts. *G. mellonella* are inexpensive to purchase and do not require specialized facilities for maintenance. The relatively large size of the larvae facilitates easy handling, injection of a defined inoculum, and sampling for downstream analyses. Furthermore, in contrast to other invertebrate hosts, *G. mellonella* larvae can be maintained at temperatures up to 37 °C, equivalent to the temperature in mammalian hosts.[1] As temperature has been shown to affect expression of *Candida* virulence traits, this feature is important when assessing virulence of *Candida* strains.[2] In addition, the larval immune system shows functional and structural similarity to the mammalian innate immune system: Pathogens are recognized by pathogen recognition receptors and can be phagocytosed by the insects' hemocytes, the functional equivalent to mammalian neutrophils. Similar to neutrophils, hemocytes use reactive oxygen species and lytic enzymes to eliminate microorganisms.[3] Antimicrobial peptides are produced by *G. mellonella* in response to infection and likely contribute to the host defense, as it has been shown for *Candida* epithelial infections using mammalian cells.[4,5] Thus, it is not surprising that *G. mellonella* larvae are used increasingly as a model for *Candida* infections, for example to determine the virulence of genetically modified *C. albicans* strains[6-11] and to determine the efficacy of antifungal treatment against both *C. albicans* and non-*albicans Candida* species.[12-16]

Host mortality after infection with a distinct dose or determination of the LD_{50} is commonly used as the primary parameter to assess virulence of microorganisms and to rank the relative virulence of species and strains. Using this approach with systemically infected mice, *C. albicans* and *C. tropicalis* were found to be highly virulent while other *Candida* species such as *C. glabrata*, *C. parapsilosis*, and *C. krusei* induced no mortality,[17] even in immunocompromised mice.[18,19] The high virulence of *C. albicans* in murine models correlates with the clinical situation, in which the majority of *Candida* infections are caused by *C. albicans*. However, infections with non-albicans *Candida* species are emerging, including species such as *C. glabrata* and *C. parapsilosis*, which rarely cause lethal infections in mice. In the absence of mortality, fungal burden can be used to compare species and strains.[17] However, fungal burden primarily reflects fitness and not necessarily virulence, as illustrated by a *C. albicans* mutant overexpressing the transcription factor *NRG1*: This mutant was highly attenuated in a systemic mouse model although fungal burden was comparable to the corresponding wild-type strain.[20] Comparison of the virulence potential of different *Candida* spp. has also been performed in *G. mellonella*, confirming *C. albicans* and *C. tropicalis* as the most virulent species.[16,21,22] In addition, these studies also revealed substantial virulence potential of *C. parapsilosis* in *G. mellonella*, leading to significant mortality of infected larvae. Although it is not clear why *C. parapsilosis* infections are lethal in *G. mellonella* larvae but not in mice, this observation suggested that *G. mellonella* could serve as a model organism to study virulence on level of subspecies and strains. Thus, Gago et al. used mortality in the *Galleria* model as the primary parameter to investigate the virulence potential of the species within in *C. parapsilosis* complex, *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis*.[23] This study, published recently in *Virulence*, showed that *C. parapsilosis* and *C. orthopsilosis* induced larval mortality at a comparable rate while *C. metapsilosis* was less virulent. These findings are strongly supported by a recent publication of Németh et al., who obtained comparable results using a different set of strains belonging to the *C. parapsilosis* complex in a *G. mellonella* infection model.[24] The results are furthermore consistent with different in vitro approaches, that found *C. metapsilosis* to be the least virulent species of the *parapsilosis* complex,[25-27] and virulence in a vaginal mouse model.[27]

Why is *C. metapsilosis* less virulent than *C. parapsilosis* and *C. orthopsilosis*? The ability to secrete proteases and lipases has

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been associated with virulence in *C. albicans* and *C. parapsilosis*.[28,29] Both Gago et al. and Németh et al. analyzed enzymatic activity in the different strains and found *C. parapsilosis* strains to more frequently express hydrolytic activity. However, as *C. orthopsilosis* and *C. parapsilosis* showed comparable virulence in the *Galleria* model, additional factors must contribute to pathogenicity. Filament formation, mediating penetration of tissue and escape from immune cells upon phagocytosis, is a well described virulence attribute in *C. albicans*.[30] Most *C. parapsilosis* and *C. orthopsilosis* strains were capable of forming pseudohyphae in vitro whereas all *C. metapsilosis* isolates analyzed produced yeast cells only, suggesting a link between pseudohyphae formation and virulence on the species level.[23,24] On the strain level, however, individual *C. orthopsilosis* isolates displayed high virulence in the absence of pseudohyphae formation, suggesting that additional factors are important. Interestingly, partial decoupling of filamentation and virulence has also been observed with defined *C. albicans* mutants in the *G. mellonella* model: A *C. albicans tec1*Δ mutant that still formed filaments exhibited reduced pathogenicity in *G. mellonella* model. Similarly, restoration of filamentation in *C. albicans flo8*Δ by overexpression of *TEC1* did not restore virulence, both in *G. mellonella* and in mice.[7] As filament formation enables *Candida* to escape from immune cells, Gago et al. analyzed the hemocytes in infected *G. mellonella* larvae. Hemocytes numbers were significantly lower in larvae infected with *C. parapsilosis* and *C. orthopsilosis* compared with *C. metapsilosis*. Correlation of hemocytes numbers with survival after *Candida* infection has also been observed in other studies[5,15,31-33] and hemocytes function has been clearly linked to the outcome of fungal infections in *Galleria*.[34,35] Gago et al. speculated that pseudohyphae production by phagocytosed yeasts may damage hemocytes, thus contributing to the lower number of hemocytes observed. This hypothesis is supported by the results of Németh et al., who demonstrated a greater cytotoxic potential of *C. parapsilosis* and *C. orthopsilosis* against mammalian macrophages.[24] However, it yet needs to be demonstrated that *Galleria* hemocytes are indeed damaged by pseudohyphae and that this process accounts for the lower number of hemocytes in vivo, and in consequence for higher virulence. In this context, the higher rate of phagocytosis of *C. metapsilosis* by *Galleria* hemocytes might indicate reduced survival of this fungus in the host, a hypothesis which likewise still needs to be experimentally confirmed.

The study of Gago et al. illustrates the potential of *G. mellonella* larvae as a model organism to assay *Candida* virulence and to study pathogenesis. However, many questions remain open, for example which kind of hemocytes respond to *Candida* infections, which fungal ligands do bind to what hemocyte receptors and whether an unbalanced immune response contributes to pathogenesis, as described in the murine model of disseminated candidiasis and human sepsis.[36] To address these questions, it will be necessary to develop tools that allow investigating different interactions on the cellular and molecular level, as they are available for mice, humans, and other model organisms, such as *Drosophila*. Useful tools could include immortalized *G. mellonella* cell lines, antibodies to allow differentiation of hemocytes populations, markers for hemocytes activation, genome data to facilitate development of microarrays and methods for genomic manipulation of *G. mellonella*. Furthermore, as recently elaborated in other editorials in *Virulence*, defined *G. mellonella* lines and standardized protocols for propagation and maintenance are needed to fully develop the potential of *G. mellonella* larvae as model organisms to study fungal infections and to allow comparison of results obtained in different laboratories.[37] A better understanding of the pathogenesis of fungal infections in *Galleria* will likely yield important insights into the infection process that can be transferred to mammalian host. There will also be limitations and it is likely that pathogenesis differs in some aspects between different host species. For example, it remains to be elucidated why infection with *C. parapsilosis* is lethal in *G. mellonella* larvae but not in mice. These differences, however, if seen in the context of human infections, should not be merely considered a disadvantage of one model over the other. If interpreted with care, understanding both similarities and differences of pathogenesis and host defense in different model hosts will greatly aid in identifying mechanisms that can be transferred to human infections. In addition, it might furthermore help in elucidating specific defects that predispose human patients to infection and possibly allow identifying new approaches for treatment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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