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EDITORIAL

Of mice and men and larvae: *Galleria mellonella* to model the early host-pathogen interactions after fungal infection

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Candida species are the most common fungal causes of deep-seated and disseminated infections in immunocompromised human hosts, and are associated with high morbidity and mortality in this patient population.[1,2] Although the incidence of invasive fungal infections caused by unusual *Candida* spp. continues to rise, to date *Candida albicans* remains the most frequently isolated *Candida* species in the clinical setting, is the principal agent of nosocomial yeast infections,[1,3] and is widely accepted as being one of the most virulent *Candida* species.[4] A large number of virulence and fitness factors have been identified in *C. albicans*, and include the ability to undergo yeast-hyphal transition, the expression of adhesins, invasins and hydrolytic enzymes which promote biofilm formation and tissue invasion, and rapid adaptations to changing extracellular environments (reviewed in[5]). However, since disease severity and outcome depend upon the complex interplay between the virulence of the individual fungal pathogen and the immune response of the host,[6] study of both aspects is central to furthering our understanding of fungal pathogenesis.

A large number of animal models have been developed to allow the study of both superficial and disseminated candidosis, and murine models continue to be promoted as the gold standard for evaluating *Candida* pathogenicity.[7] Study of the host response and the determinants of fungal virulence have been aided by such animal models since they replicate human disease with high fidelity. In addition, the availability of genetically modified or immune-depleted hosts has allowed the dissection of the principal host immune components.[8] However, animal experimentation is constrained by major bioethical, economic and logistical issues, and the "3Rs" policy adopted by many international and governmental funding agencies encourages the development of alternative model systems that do not have associated bioethical issues. Amongst alternative model systems for studying microbial pathogenesis, two insects in particular have been extensively used over recent years: the fruit fly *Drosophila melanogaster* and the larvae of the greater wax moth *Galleria mellonella* (reviewed in[9]). Advantages include inexpensive and easy breeding in large numbers, relatively simple maintenance in the laboratory, and ease of inoculation. In addition, insect innate immune systems at the cellular and humoral level are structurally and functionally very similar to the immune system in mammals, allowing results obtained in insect models to be easily translated to humans.[10,11] In insects, pathogens are recognised by pathogen recognition receptors,[12,13] phagocytosed by hemocytes which are functional equivalents of mammalian neutrophils,[14] and eliminated using reactive oxygen species as in mammals.[15] Insects also synthesise a range of antimicrobial peptides, many of which are evolutionarily conserved between invertebrates and mammals.[16,17]

The ability to infect *Drosophila* with fungi, bacteria and viruses,[18] the availability of a completed genome sequence[19] and extensive gene microarrays[20] and the ease of genetic manipulation have made this organism a model system for infection studies.[21] Indeed, research with *Drosophila* has elucidated many of the central mechanisms of anti-pathogen immunity,[21,22] including the discovery that the Toll signalling pathway was central to an effective antifungal host response against *Aspergillus fumigatus*.[23] However, despite the lack of equivalent genomic resources, *Galleria* also has specific advantages for the study of human pathogens and is an increasingly

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popular choice for the investigation of the determinants of fungal pathogenesis. Wild-type larvae are susceptible to human-pathogenic fungi without the need for manipulation of the Toll pathway and genetic crossing, and can be housed in simple petri dishes in the laboratory. The larger size of the larvae as compared to *Drosophila* potentially allows multiple inoculations into the same organism, the use of more accurately quantified inocula with less specialised equipment, and more sophisticated and varied end-point analyses (melanisation, fungal burden, larval death, alterations in hemocyte composition and larval genomic/proteomic changes).[24,25] Moreover, *Galleria* larvae can be maintained at human physiological temperatures and above, an important consideration when temperature-dependent virulence factors are involved.[26,27] In addition, several studies have demonstrated that pre-exposure of *G. mellonella* to sub-lethal doses of fungi protected the larvae from subsequent lethal challenges, in part by inducing the production of protective antimicrobial peptides, indicating that *Galleria* is able to assess the extent of fungal infection and differentially activate cellular and humoral responses.[28,29] To date, *Galleria mellonella* has been successfully employed in studies comparing virulence of different fungi,[30,31] elucidation of virulence factors,[32-34] antifungal drug response and resistance,[35-37] combination therapy[38] and pharmacokinetics,[39] alternative antifungal therapies[40,41] and probiotics.[42,43] In addition, this invertebrate model is apparently capable of reproducing clinical features seen with human infection with remarkable fidelity, as evidenced by the demonstration of fungal grain development in larvae infected with an agent of eumycetoma[44] and aggregates of immobilised *Listeria* bacteria on the brains of larvae that are similar to those seen on the brains of infected humans.[45]

In a recent issue of *Virulence*,[46] the group of Kavanagh, one of the leading proponents of the use of *Galleria* as a fungal infection model has further strengthened the argument, by exploiting the recently published transcriptome and immune-gene repertoire of *Galleria*[47] to examine the early cellular and humoral responses of larvae after infection by *Candida albicans*. The authors infected larvae with a dose of *C. albicans* that permitted larval survival for 24 hours and followed fungal burden, changes in hemocyte numbers and population structure and employed semi-quantitative shotgun proteomics to analyse the larval response early (6 hours) and late (24 hours) after infection. Their analyses revealed a biphasic response to infection. In the early acute phase, hemocyte density, antimicrobial peptides and immune proteins all increased significantly in abundance with a concomitant reduction in larval fungal burden. The late phase (6-24 hours) conversely was marked by extensive fungal proliferation, reduction of the overall hemocyte population with an increase in the number of granular hemocytes and proteomic changes indicative of cellular stress, tissue damage, chemo-protection and sequestration of key immune related proteins in immune-fungal complexes. These data are highly suggestive of a sophisticated and orchestrated response in which early stages are designed to gauge the extent of fungal infection via non-specific immune reactions (antimicrobial peptides, melanisation) and determine the full immune response required, followed by the later unleashing of a large specific response involving increased production of antimicrobial peptides and phagocytic cells. What makes the study more important is that the initial cellular and humoral responses observed in *Galleria* appear very similar to those that are seen in murine models of invasive candidiasis, where an initial massive innate immune response involving cellular recruitment, complement activation and opsonisation occurs early in the neutrophil-poor kidneys of infected animals prior to wide scale peripheral neutrophilia.[48] Moreover, this latest addition to the *Galleria* literature clearly demonstrates that the lack of whole genome data for this model insect does not prevent a detailed exploration of host-pathogen interactions.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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References

1. Marr KA. Invasive *Candida* infections: the changing epidemiology. Oncology. 2004;14:9–14
2. Nucci M, Marr KA. Emerging fungal diseases. Clin Infect Dis. 2005;41:521–526. doi:10.1086/432060. PMID:16028162
3. Ruhnke M. Epidemiology of *Candida albicans* infections and role of non-*Candida albicans* yeasts. Curr Drug Targets. 2006;7:495–504. doi:10.2174/138945006776359421. PMID:16611037
4. Borman AM, Szekely A, Johnson EM. Comparative pathogenicity of United Kingdom isolates of the emerging pathogen *Candida auris* and other key pathogenic *Candida* species. mSphere. 2016;1(4):e00189–16. doi:10.1128/mSphere.00189-16. PMID:27547827
5. Mayer FL, Wilson D, Hube B. *Candida albicans* pathogenicity mechanisms. Virulence. 2013;4(2):119–28. doi:10.4161/viru.22913. PMID:23302789
6. Casadevall A, Pirofski LA. What is a host? Incorporating the microbiota into the damage-response framework. Infect Immun. 2015;83(1):2–7 doi:10.1128/IAI.02627-14. PMID:25385796

7. Conti HR, Huppler AR, Whibley N, Gaffen SL. Animal models for candidiasis. Curr Protoc Immunol. 2014;105:19.6.1–17. doi:10.1002/0471142735.im1906s105.
8. Sparber F, LeibundGut-Landmann S. Interleukin 17-mediated host defence against *Candida albicans*. Pathogens. 2015;4(3):606–19. doi:10.3390/pathogens4030606. PMID:26274976
9. Lionakis MS. *Drosophila* and *Galleria* insect model hosts: new tools for the study of fungal virulence, pharmacology and immunology. Virulence. 2011;2(6):521–7. doi:10.4161/viru.2.6.18520. PMID:22186764
10. Kavanagh K, Reeves EP. Exploiting the potential of insects for *in vivo* pathogenicity testing of microbial pathogens. FEMS Microbiol Rev. 2004;28(1):101–12. doi:10.1016/j.femsre.2003.09.002. PMID:14975532
11. Brennan M, Thomas DY, Whiteway M, Kavanagh K. Correlation between virulence of *Candida albicans* mutants in mice and *Galleria mellonella* larvae. FEMS Immunol Med Microbiol. 2002;34(2):153–7. doi:10.1111/j.1574-695X.2002.tb00617.x. PMID:12381467
12. Stokes BA, Yadav S, Shokal U, Smith LC, Eleftherianos I. Bacterial and fungal pattern recognition receptors in homologous innate signaling pathways of insects and mammals. Front Microbiol. 2015;6:19. doi:10.3389/fmicb.2015.00019. PMID:25674081
13. Kim CH, Shin YP, Noh MY, Jo YH, Han YS, Seong YS, Lee IH. An insect multiligand recognition protein functions as an opsonin for the phagocytosis of microorganisms. J Biol Chem. 2010;285(33):25243–50. doi:10.1074/jbc.M110.134940. PMID:20519517
14. Browne N, Heelan M, Kavanagh K. An analysis of the structural and functional similarities of insect hemocytes and mammalian phagocytes. Virulence. 2013;4(7):597–603. doi:10.4161/viru.25906. PMID:23921374
15. Bergin D, Reeves EP, Renwick J, Wientjes FB, Kavanagh K. Superoxide production in *Galleria mellonella* hemocytes: identification of proteins homologous to the NADPH oxidase complex of human neutrophils. Infect Immun. 2005;73(7):4161–70
16. Bergman P, Seyedoleslami Esfahani S, Engström Y. *Drosophila* as a model for human diseases- focus on innate immunity in barrier epithelia. Curr Top Dev Biol. 2017;121:29–81. doi:10.1016/bs.ctdb.2016.07.002. PMID:28057304
17. Yi HY, Chowdhury M, Huang YD, Yu XQ. Insect antimicrobial peptides and their applications. Appl Microbiol Biotechnol. 2014;98(13):5807–22. doi:10.1007/s00253-014-5792-6. PMID:24811407
18. Panayidou S, Ioannidou E, Apidianakis Y. Human pathogenic bacteria, fungi, and viruses in *Drosophila*: disease modeling, lessons, and shortcomings. Virulence. 2014;5(2):253–69. doi:10.4161/viru.27524. PMID:24398387
19. Lin SC, Chang YY, Chan CC. Strategies for gene disruption in *Drosophila*. Cell Biosci. 2014;4(1):63. doi:10.1186/2045-3701-4-63. PMID:25364499
20. Gupta V, Oliver B. *Drosophila* microarray platforms. Brief Funct Genomic Proteomic. 2003;2(2):97–105. doi:10.1093/bfgp/2.2.97. PMID:15239930
21. Tzou P, De Gregorio E, Lemaitre B. How *Drosophila* combats microbial infection: a model to study innate immunity and host-pathogen interactions. Curr Opin Microbiol. 2002; 5(1):102–10. doi:10.1016/S1369-5274(02)00294-1. PMID:11834378
22. Imler JL. Overview of *Drosophila* immunity: a historical perspective. Dev Comp Immunol. 2014;42(1):3–15. doi:10.1016/j.dci.2013.08.018. PMID:24012863
23. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell. 1996;86(6):973–83. doi:10.1016/S0092-8674(00)80172-5. PMID:8808632
24. Fallon J, Kelly J, Kavanagh K. Methods. *Galleria mellonella* as a model for fungal pathogenicity testing. Mol Biol. 2012;845:469–85
25. Bergin D, Brennan M, Kavanagh K. Fluctuations in haemocyte density and microbial load may be used as indicators of fungal pathogenicity in larvae of *Galleria mellonella*. Microbes Infect. 2003; 5(15):1389–95. doi:10.1016/j.micinf.2003.09.019. PMID:14670452
26. Mowlds P, Kavanagh K. Effect of pre-incubation temperature on susceptibility of *Galleria mellonella* larvae to infection by *Candida albicans*. Mycopathologia. 2008; 165(1):5–12. doi:10.1007/s11046-007-9069-9. PMID:17922218
27. Borman AM, Szekely A, Linton CJ, Palmer MD, Brown P, Johnson EM. Epidemiology, antifungal susceptibility, and pathogenicity of *Candida africana* isolates from the United Kingdom. J Clin Microbiol. 2013; 51(3):967–72. doi:10.1128/JCM.02816-12. PMID:23303503
28. Bergin D, Murphy L, Keenan J, Clynes M, Kavanagh K. Pre-exposure to yeast protects larvae of *Galleria mellonella* from a subsequent lethal infection by *Candida albicans* and is mediated by the increased expression of antimicrobial peptides. Microbes Infect. 2006;8(8):2105–12
29. Fallon JP, Troy N, Kavanagh K. Pre-exposure of *Galleria mellonella* larvae to different doses of *Aspergillus fumigatus* conidia causes differential activation of cellular and humoral immune responses. Virulence. 2011;2(5):413–21. doi:10.1016/j.micinf.2006.03.005. doi:10.4161/viru.2.5.17811. PMID: 21921688. PMID:16782387
30. Binder U, Maurer E, Lass-Flörl C. *Galleria mellonella*: An invertebrate model to study pathogenicity in correctly defined fungal species. Fungal Biol. 2016;120(2):288–95. doi:10.1016/j.funbio.2015.06.002. PMID:26781383
31. Cotter G, Doyle S, Kavanagh K. Development of an insect model for the in vivo pathogenicity testing of yeasts. FEMS Immunol Med Microbiol. 2000;27(2):163–9. doi:10.1111/j.1574-695X.2000.tb01427.x. PMID:10640612
32. Trieu TA, Navarro-Mendoza MI, Pérez-Arques C, Sanchis M, Capilla J, Navarro-Rodriguez P, Lopez-Fernandez L, Torres-Martínez S, Garre V, Ruiz-Vázquez RM, et al. RNAi-based functional genomics identifies new virulence determinants in Mucormycosis. PLoS Pathog. 2017;13(1):e1006150. doi:10.1371/journal.ppat.1006150. PMID:28107502
33. 33 Renshaw H, Vargas-Muñiz JM, Richards AD, Asfaw YG, Juvvadi PR, Steinbach WJ. Distinct roles of myosins in *Aspergillus fumigatus* hyphal growth and pathogenesis. Infect Immun. 2016; 84(5):1556–64. doi:10.1128/IAI.01190-15. PMID:26953327
34. 34 Moralez AT, Perini HF, Furlaneto-Maia L, Almeida RS, Panagio LA, Furlaneto MC. Phenotypic switching of *Candida tropicalis* is associated with cell damage in epithelial cells and virulence in *Galleria mellonella* model. Virulence. 2016;7(4):379–86. doi:10.1080/21505594.2016.1140297. PMID:26760314

35. 35 Fuchs BB, Li Y, Li D, Johnston T, Hendricks G, Li G, Rajamuthiah R, Mylonakis E. Micafungin elicits an immunomodulatory effect in *Galleria mellonella* and mice. Mycopathologia. 2016;181(1-2):17–25. doi:10.1007/s11046-015-9940-z. PMID:26384671
36. Souza AC, Fuchs BB, Pinhati HM, Siqueira RA, Hagen F, Meis JF, Mylonakis E, Colombo AL. *Candida parapsilosis* resistance to fluconazole: molecular mechanisms and *in vivo* impact in infected *Galleria mellonella* larvae. Antimicrob Agents Chemother. 2015;59(10):6581–7. doi:10.1128/AAC.01177-15. PMID:26259795
37. Scorzoni L, de Lucas MP, Mesa-Arango AC, Fusco-Almeida AM, Lozano E, Cuenca-Estrella M, Mendes-Giannini MJ, Zaragoza O. Antifungal efficacy during *Candida krusei* infection in non-conventional models correlates with the yeast *in vitro* susceptibility profile. PLoS One. 2013;8(3):e60047. doi:10.1371/journal.pone.0060047. PMID:23555877
38. Mylonakis E, Moreno R, El Khoury JB, Idnurm A, Heitman J, Calderwood SB, Ausubel FM, Diener A. *Galleria mellonella* as a model system to study *Cryptococcus neoformans* pathogenesis. Infect Immun. 2005;73(7):3842–50. doi:10.1128/IAI.73.7.3842-3850.2005. PMID:15972469
39. Astvad KMT, Meletiadis J, Whalley S, Arendrup MC. Fluconazole pharmacokinetics in the *Galleria mellonella* larvae and performance evaluation of a bioassay compared to LC-MS/MS for haemolymph specimens. Antimicrob Agents Chemother. 2017;61(10). pii: e00895–17. doi:10.1128/AAC.00895-17. PMID:28760893
40. de Oliveira HC, Michaloski JS, da Silva JF, Scorzoni L, de Paula E, Silva AC, Marcos CM, Assato PA, Yamazaki DS, Fusco-Almeida AM, et al. Peptides derived from a phage display library inhibit adhesion and protect the host against Infection by *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii*. Front Pharmacol. 2016;7:509. doi:10.3389/fphar.2016.00509. PMID:28066254
41. Sangalli-Leite F, Scorzoni L, Alves de Paula E, Silva AC, da Silva JF, de Oliveira HC, de Lacorte Singulani J, Gullo FP, Moraes da Silva R, Regasini LO, et al. Synergistic effect of pedalitin and amphotericin B against *Cryptococcus neoformans* by *in vitro* and *in vivo* evaluation. Int J Antimicrob Agents. 2016;48(5):504–511. doi:10.1016/j.ijantimicag.2016.07.025. PMID:27742203
42. Rossoni RD, Fuchs BB, de Barros PP, Velloso MD, Jorge AO, Junqueira JC, Mylonakis E. *Lactobacillus paracasei* modulates the immune system of *Galleria mellonella* and protects against *Candida albicans* infection. PLoS One. 2017; 12(3):e0173332. doi:10.1371/journal.pone.0173332. PMID:28267809
43. Ribeiro FC, de Barros PP, Rossoni RD, Junqueira JC, Jorge AO. *Lactobacillus rhamnosus* inhibits *Candida albicans* virulence factors in vitro and modulates immune system in *Galleria mellonella*. J Appl Microbiol. 2017;122(1):201–211. doi:10.1111/jam.13324. PMID:27727499
44. Kloezen W, van Helvert-van Poppel M, Fahal AH, van de Sande WW. A *Madurella mycetomatis* grain model in *Galleria mellonella* larvae. PLoS Negl Trop Dis. 2015; 9(7):e0003926. doi:10.1371/journal.pntd.0003926. PMID:26173126
45. Mukherjee K, Hain T, Fischer R, Chakraborty T, Vilcinskas A. Brain infection and activation of neuronal repair mechanisms by the human pathogen *Listeria monocytogenes* in the lepidopteran model host *Galleria mellonella*. Virulence. 2013; 4(4):324–32 doi:10.4161/viru.23629. PMID:23348912
46. Sheehan G, Kavanagh K. Analysis of the early cellular and humoral responses of *Galleria mellonella* larvae to infection by *Candida albicans*. Virulence. 2017 doi:10.1080/21505594.2017.1370174. PMID:28872999
47. Vogel H, Altincicek B, Glöckner G, Vilcinskas A. A comprehensive transcriptome and immune-gene repertoire of the lepidopteran model host *Galleria mellonella*. BMC Genomics. 2011;12:308. PMID:21663692
48. MacCallum DM. Massive induction of innate immune response to Candida albicans in the kidney in a murine intravenous challenge model. FEMS Yeast Res. 2009; 9:1111–22.