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Galleria mellonella as a model host for human pathogens

Recent studies and new perspectives

Juliana Campos Junqueira

Department of Biosciences and Oral Diagnosis; São José dos Campos Dental School; Universidade Estadual Paulista/UNESP; São Paulo, Brazil

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The number of studies using *G. mellonella* as a model host for human pathogens has increased significantly in the last few years. Important studies were published from different countries for evaluating the pathogenesis of bacterial and fungal infections and for exploring the host defenses against pathogens. Therefore, standardized conditions for the use of *G. melonella* larvae need to be established. Recent research showed that the deprivation of *G. mellonella* larvae of food during the experiment caused a reduction in immune responses and an increased susceptibility to infection, suggesting that incubating of larvae in the presence or absence of nutrition may affect the results and comparisons among different laboratories.

Larvae of the greater wax moth *Galleria mellonella* have recently been used as model hosts for studying pathogenic microorganisms as an alternative to vertebrates. A positive correlation between virulence and host response has generally been found in both invertebrate and mammalian host models for a range of microorganisms, such as *Acinetobacter baumanii*,[1,2] *Francisella tularensis*,[3] *Pseudomonas aeruginosa*,[4,5] *Yersinia pseudotuberculosis*,[6] *Staphylococcus aureus*,[7] *Streptococcus pyogenes*,[8] *Streptococcus mutans*,[9] *Enterococcus faecalis*,[10,11] *Candida albicans*[12] and *Cryptococcus neoformans*.[13]

In 2010, Fuchs and colleagues[14] reported in *Virulence* several methods for using *Galleria mellonella* as a model host to study fungal pathogenesis. First, these authors described a number of the benefits of using *G. mellonella* larvae as a model host that are not easily achieved with invertebrate models such as *Caenorhabditis elegans* and *Drosophila melanogaster*. For example, the larvae of *G. mellonella* can be maintained at 37°C. This characteristic is very important, because it allows microorganisms to be studied under the temperature conditions at which they are pathogenic to human hosts. Another benefit of the *G. mellonella* model is the multiple options for facile delivery of the pathogen, such as topical application, oral delivery and injection. Among these methods, injection offers the benefit that fungi can be injected directly into the larval hemocoel and therefore larvae receive a known amount of pathogens. Moreover, the *G. mellonella* model is not restricted to studies that examine aspects of the pathogenesis of fungal infections but also recommends itself to the study of host defenses against fungal pathogens. *G. mellonella* have an innate immune system comprised of different types of hemocytes, which play a role in fungal-pathogen defense.

Next, Fuchs et al.[14] presented in detail various methods to study fungal virulence and the association of fungal cells with insect hemocytes using *Candida albicans* and *Cryptococcus neoformans* to illustrate the use of this model. These authors showed that *G. mellonella* can be used to monitor fungal pathogenicity by a survival assay. Larvae can also be utilized to observe differences in fungal cell filamentation post-infection. For this experiment, the fat body and other internal structures of *G. mellonella* can be collected, fixed with formalin, and prepared for histological sectioning. Furthermore, the authors demonstrated how fungal cell-hemocyte associations can be evaluated using fluorescence-activated cell sorting (FACS) analysis. The study by Fuchs et al.[14] has great value to the scientific community, because the protocols presented for the *G. mellonella* infection model can be adapted to the study of other fungal and bacterial pathogens.

In this context, Olsen and colleagues[8] in 2011 published in *Virulence* the first study to describe *G. mellonella* as model host for group A streptococcus (GAS, *S. pyogenes*). To test the hypothesis that *G. mellonella* is a suitable model host to study GAS pathogenesis, the authors infected larvae with serotype M3 strain MGAS315. The genome of this strain has been sequenced, and it is representative of highly virulent serotype M3 GAS strains that cause severe invasive disease in humans. In addition, strain MGAS315 has been extensively studied in previous experiments using mice and monkeys. All larvae infected with strain MGAS315 had distinct signs of

Correspondence to: Juliana Campos Junqueira; Email: juliana@fosjc.unesp.br
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invasive infection, including melanization, rapid death and formation of a destructive abscess-like lesion at the site of inoculation. These abscesses comprised a dense central core of necrotic tissue and GAS microorganisms surrounded by a well-organized outer band of host hemocytes, coagulated hemolymph and extracellular melanin pigment. According to the authors, these findings are similar to the histopathology that is commonly observed in mouse and monkey models of GAS necrotizing fasciitis and in humans with severe soft tissue infections. Therefore, these results showed that *G. mellonella* larvae are useful host organisms for studing GAS pathogenesis.

In the same year, *Virulence* published another interesting study related to the *G. mellonella* model, in which this insect's immune response to infection was extensively explored by Fallon and colleagues.[15] In this study, the authors demonstrated that prior exposure of *G. mellonella* larvae to non-lethal doses (1×10^4 or 1×10^5) of *Aspergillus fumigatus* conidia increased the larval survival rate when a lethal dose (1×10^7) was administered 24 h later, suggesting that the inoculation of *G. mellonella* with non-lethal doses of *A. fumigatus* conferred a significant protective response against a subsequent lethal inoculum. According to Fallon et al.,[15] insects do not have an immune system that is analogous to the adaptative immune response of mammals in terms of antibody generation, but they do have the capacity to mount an immune response in anticipation of a subsequent infection that has some elements that are similar to the function of the adaptive immune response in mammals. This study significantly contributes to research exploring *G. mellonella* as a model host, because an understanding of the mechanisms employed by insects to withstand infection is critical to their successful use as models for human pathogens.

Thus, we have observed that *G. mellonella* as a model for the study of infectious diseases has achieved increasing acceptance among scientific researchers, and the use of this invertebrate model in medical research extends to many laboratories around the world. Recently, important studies were published from different countries, such as the US,[12,16,17] Ireland,[15,17,18] Canada,[19] the United Kingdom,[20] Spain,[21] Germany,[22,23] Brazil,[24] Tunisia,[25] Greece,[26] South Korea,[27] Poland,[28] Italy[29] and Norway.[30] Therefore, studies need to be developed to determine standardized conditions for the propagation and maintenance of *G. mellonella* larvae.

In this issue of *Virulence*, Banville and colleagues[31] have published a study to evaluate the effect of nutritional deprivation on the ability of larvae to withstand infection. The objective of this study was to establish standardized conditions for larval treatment for in vivo testing, given that some researchers incubate larvae with a food source during experiments, while others do not. The authors observed that larvae deprived of nutrition for 7 days demonstrated increased susceptibility to infection with the fungal pathogen *C. albicans*. Starved larvae demonstrated a slight reduction in hemocyte density, but the hemocytes from starved larvae were as effective at killing *C. albicans* cells as those from unstarved larvae. Hemolymph from starved larvae showed reduced expression of a range of antimicrobial peptides and immune proteins. Banville et al.[31] concluded that the deprivation of *G. mellonella* larvae of food leads to a reduction in cellular and immune responses and an increased susceptibility to infection, indicating that researchers utilizing *G. mellonella* for the study of human pathogens should specify whether food is provided to the larvae to allow valid comparisons between results from different laboratories.

According to the studies cited above, it is evident that the number of studies using *G. mellonella* as a model host has increased significantly in the last few years. In addition, there has been an improvement in the techniques used with this model, which allows further possibilities for the development of other studies. Certainly, the articles published in *Virulence* represent an important scientific contribution for the advancement of research utilizing *G. mellonella* as a model host for human pathogens.

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