Virulence 4:4, 271–272; May 15, 2013; © 2013 Landes Bioscience

Developing the potential of using *Galleria mellonella* larvae as models for studying brain infection by *Listeria monocytogenes*

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Keywords: brain, *Galleria*, *Listeria*, immunity, in vivo model, virulence

The use of mammals for studying microbial pathogens or for assessing the efficacy of a variety of pharmaceutical agents has contributed enormously to our knowledge of microbial virulence and in the development of novel therapeutic strategies. However, despite these advances, there is a recognition that the number of mammals used in such tests must be reduced to the minimum and that alternative, but ethically acceptable, systems must be developed. This has led to the adoption of the 3R policy by many government and international funding agencies in an effort to encourage the development of more acceptable but scientifically valid screening systems. A variety of models have been developed as alternative systems in recent years including cell culture, tissue explants and nematodes. Insects such as *Drosophilia melanogaster* (fruit fly), *Galleria mellonella* (wax worm), and *Bombyx mori* (silk worm) have become attractive alternative models for studying host pathogen interactions and for assessing the efficacy of novel antimicrobials.[1-4] The immune system of insects is very similar structurally and functionally to the innate immune response of mammals hence results obtained using insects can readily be translated to mammals.[1] Insects offer a number of advantages as models including high throughput (24–48 h),[5] ease of inoculation, a lack of ethical restrictions and most can be cultured easily or purchased relatively inexpensively.[4,6]

The insect cuticle has a similar function to the skin of mammals and acts as the first line of defense by retarding pathogen entry and may also contain antimicrobial compounds.[7] The insect hemolymph, which is held within the body cavity (hemocoel), functions in a similar manner to the blood of mammals by transporting nutrients, signal molecules, antimicrobial peptides (AMP), immune cells, and waste products.[8] The insect cellular immune response is mediated by hemocytes, which demonstrate a range of functional and structural similarities to mammalian neutrophils—both can phagocytose and neutralize engulfed pathogens through the generation of superoxide and the secretion of lytic enzymes in the process known as degranulation.[9] Insect humoral immune responses to eliminate pathogens also display similar mechanisms to those observed in the mammalian humoral immune response. The melanization process in insects occurs in a similar manner to mammals via the use of complement proteins and both insects and mammals use similar pathogen recognition receptors (PRRs) to identify and opsonize pathogenic surfaces. PRRs and signaling cascades such as the Toll-like receptor pathways demonstrate a high degree of conservation between the mammalian and insect immune signaling pathways.[10] Antimicrobial peptides that are generated within the insect fat body, which is equivalent in function to the mammalian liver, can also be synthesized by hemocytes and at environmentally exposed regions of the respiratory and genital tracts of insects.[1]

Although insects lack the adaptive immune system of vertebrates they have been widely used to study the virulence of microbial pathogens and to assess the efficacy of antimicrobial drugs due to the common cellular and humoral functions seen in both insect and mammal innate immune systems.[1,4] *G. mellonella* larvae are widely used as in vivo models to screen pathogens and give results within 24–48 h. Larvae may be incubated at 30–37 °C enabling the study of temperature-dependent virulence factors.[11] Assessment of the virulence of microbial pathogens or the efficacy of antimicrobial agents can be assessed by monitoring a number of parameters in *G. mellonella* including the degree of melanization in response to a pathogen, larval death, alterations in fungal burden, alterations in hemocyte densities and/or function, alteration in gene expression or internal proteome.[12-14]

The use of *G. mellonella* larvae in the assessment of fungal virulence has been demonstrated using *Candida albicans*,[5,15] *Aspergillus fumigatus*,[16] and *Cryptococcus neoformans*.[17,18] A variety of bacterial pathogens, including *Listeria monocytogenes*[19,20] and *Bacillus* species,[21,22] have also been screened in *G. mellonella* larvae.

G. mellonella larvae have been used to evaluate the efficacy of amphotericin B, fluconazole, and flucytosine for the treatment of *C. neoformans* infection.[17] The larvae have also been used to assess the in vivo activity of novel silver-based compounds both for their antimicrobial effects and immune priming ability.[23] In addition, *Drosophila* have been utilized as a high-throughput screening system

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Submitted: 03/01/13; Accepted: 03/01/13
http://dx.doi.org/10.4161/viru.24174
Editorial to: Mukherjee K, et al. Virulence 2013; 4:324-32; PMID:23348912; http://dx.doi.org/10.4161/viru.23629

for the identification of antifungal agents effective against *Aspergillus* infection.[2] A recent study that examined the ability of caspofungin to retard *C. albicans* infection in *G. mellonella* larvae demonstrated the ability of the drug to prime the immune response of the insect.[24] This is a significant finding since it illustrated that administration of agents with no inherent antimicrobial properties (e.g., glucan) will trigger a protective immune response capable of killing bacteria and fungi.[25] This indicates that when using *Galleria* researchers must differentiate between the in vivo anti-microbial effects of an agent and also the immune priming effect.

The work of Mukherjee et al. in this issue represents a highly significant advance in the potential use of *G. mellonella* larvae.[26] In their paper the authors describe the use of *G. mellonella* larvae as a model for studying *Listeria* infection and demonstrate the production of antimicrobial peptides with anti-*Listeria* activity. Significantly, they also recorded the presence of melanized cellular aggregates (nodules) containing immobilized bacteria on the surface of the brains of larvae. Significantly, similar structures are evident on the brain of humans infected with *Listeria*. The ability of inhibitors such as diclofenac, arachidonic acid, and rapamycin to disrupt the development of *Listeria* infection and inhibit the formation of neural nodules was demonstrated. Using transcriptomic analysis they demonstrated the modulation of a variety of genes in infected larvae involved in neuronal repair and response to stress.

While insects and *G. mellonella* larvae, in particular, have been widely exploited in recent years for studying the virulence of microbial pathogens and for assessing the efficacy of antimicrobial agents, the work of Mukherjee et al. represents an important extension to the range of applications in which larvae can be employed.[26] For the first time a similar neural pathology has been demonstrated in insects as evident in humans thus opening the possibility of examining neural disease and repair mechanisms in insects. In addition these findings should enable the use of *G. mellonella* larvae for studying brain development and for rapidly evaluating the efficacy of novel drugs designed to counter a variety of neural diseases or malfunction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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