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A POWERFUL *IN VIVO* ALTERNATIVE MODEL IN SCIENTIFIC RESEARCH: *GALLERIA MELLONELLA*

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Murine models are suggested as the gold standard for scientific research, but they have many limitations of ethical and logistical concern. Then, the alternative host models have been developed to use in many aspects especially in invertebrate animals. These models are selected for many areas of research including genetics, physiology, biochemistry, evolution, disease, neurobiology, and behavior. During the past decade, *Galleria mellonella* has been used for several medical and scientific researches focusing on human pathogens. This model commonly used their larvae stage due to their easy to use, non-essential special tools or special technique, inexpensive, short life span, and no specific ethical requirement. Moreover, their innate immune response close similarly to mammals, which correlate with murine immunity. In this review, not only the current knowledge of characteristics and immune response of *G. mellonella,* and the practical use of these larvae in medical mycology research have been presented, but also the better understanding of their limitations has been provided.

Keywords: *Galleria mellonella*, innate immune response, melanization

Introduction

During the past decade, several medical and scientific researches have used wide variety of *in vivo* model organisms to address biological questions. It has been statistically surveyed that a large number of *in vivo* models are experimentally investigated worldwide each year. These animals include invertebrates (yeasts, worms, flies, etc.) and vertebrates (mice, rats, primates, etc.) [1]. In 2001, 2.13 million animals have been studied in Germany. In 2009, the number of animal models that have been used in the USA approximately is 1.13 million [2].

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In the UK, the animals used for experimental study were 3.71 and 4.87 million in 2011 and 2014, respectively [3]. Although murine models exhibit several advantages as they possess similarities to human such as anatomical structures and physiological systems, many limitations can be found in these models in terms of ethical and logistical issues, which can cause longer investigating periods than other invertebrate models [4]. The use of invertebrate models will provide an alternative choice to study the hypothesized biological questions [5–7]. Therefore, this review aims to purpose a feasible model using invertebrate animals to investigate biological studies.

Several studies have shown that the invertebrate animals are evolutionary separated from mammals thousand years ago [8]. Notably, invertebrate models do not exhibit adaptive immune system, which may limit the immunological studies in some conditions [4, 9]. However, numerous benefits are found in invertebrate models including low cost, easy to use, small size, simple anatomical structures, and short lifespan. Thus, it is an ideal model for studying in large-scale research [4].

Invertebrate models that are commonly used in scientific studies are nematodes and insects [10–13]. However, some invertebrate models cannot survive at 37 °C and lack of some functional cellular components. Among the insects available, *Galleria mellonella* has been emerged at the forefront studies [4, 14]. Over the past few years, *G. mellonella* has been used in research than other models, which showed the results correlated with mammalian models [15–19].

Galleria mellonella

G. mellonella (honeycomb moth) is a moth from the order Lepidoptera and the family Pyralidae (snout moths) [20]. The moths can be found worldwide including Europe and adjacent Eurasia, especially in the mountain range nature [21–32]. Different continents will have different species. In North America and Australia, these worms are found predominantly as the lesser wax moth (*Achroia grisella*) that is derived from the tribe Galleriini. *G. mellonella* larvae live in beehives, inside bee nests, and feed with beeswax and pollen, which are especially found mostly in weakened hives with small populations. Wax moths are very destructive insect pest in the beehive. They can cause deteriorate effects to beehives, which can destroy brood comb and woodenware. The moths can cause shed skins of bees, reduction of beeswax, and may kill the larvae or spread the honeybee diseases [33–43].

Characteristics, Anatomical, and Behavior of *G. mellonella*

G. mellonella lives in beehives and feeds with beeswax during larval stage. The larvae can also be found in bumblebee nest and wasp nest or feed on dried fig trees.

The life cycle of the wax moth consists of four definable stages, such as egg, larva, pupa, and adult. Duration of the completed cycle is approximately 6–8 weeks at 29–39 °C with 4–6 generations per year [36, 44–47].

The greater wax moth's life cycle begins with the eggs that have been placed inside the honeycomb. Olive-shaped eggs (50–150 eggs) with pink–white salmon color are placed between the cracks of the honeycomb [36, 40, 44–46, 48]. After 5–8 days, hatching of eggs and larvae will begin to develop at midrib. During the larval stage, the worm is 16–20 mm in length and resembles a caterpillar. Changes in the embryonic stage can take about 29 days at 30–35 °C, which is the optimum temperature in the nest for worm growth. Worms at low temperatures grow slower. Moreover, worm growth could be limited when worm is cultured at 4–5 °C. Thus, it could be suggested that temperature is one of the factors that plays a vital role in larval growth [34, 36, 37, 40, 42, 44–46, 49, 50].

The body of a caterpillar looks a tube-like structure for processing and storing food. The intestine is longer with the fore and hind parts (Figure 1). The worm's digestive system can be found in the fat layer called body fat. Worms produce silk using salivary glands from a tube in the spinneret, which looks like a tube on a labium (larva's lower lip) and contains the spinning apparatus (the silk glands; Figure 1). This silk is used to make cocoons and usually dried when

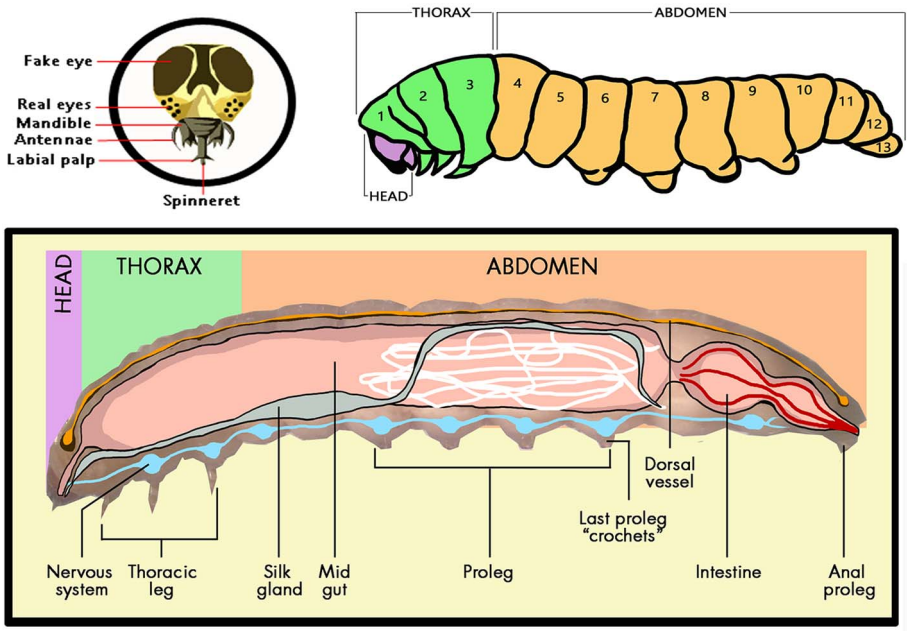

Figure 1. The anatomical model of *G. mellonella*

exposed to air. Caterpillars have a dull reddish-brown dome head, six legs at the thorax, and additional pairs of prolegs at the abdomen. These prolegs are at the terminal end of their body, which are small and hook-like called crochets (Figures 4 and 5). The skin color changes slightly to a light gray as they age. The grub eats beeswax and needs additional proteins from bee cocoons, feces, and pollen. Then, the larvae build a fine silken tube as tunnel covers them [36, 40, 42–44, 51, 52].

At the end of pupation, caterpillars start rotating the coarser silk cocoon and stop feeding, which makes the papery stronger. The development in this process takes about 4 days. The color of the cocoon depends on the supplement. If it is made of softwood, the color will be white as usual. However, the cocoon is brown when the worm is fed with hardboard (mansonite). The worm transforms into pupae in a light golden brown cocoon. The silkworm is trapped in the honeycomb cell. About 1–2 weeks, the pupae will grow into a cigar-shaped worm with 15 mm in length and will develop into adult in approximately 12 days depending on the temperature (Figure 2). Smith [42] found that there are two types of pupa, which can be distinguished between male and female. First, the border of the mesowing is creased in the upper margin of the male and straight in the female. Second, sclerite of the eighth abdominal sternum is cloven in the female but not in the male [36, 40, 43–44, 46].

Adult wax moths do not feed food during adulthood. *G. mellonella* can survive at both night and day (in a low light area) because it is reluctant to light exposure. Hence, they fly mostly at nighttime and stay in the dark during the daytime. Adult moths have 14–38 mm in length between wingspan, depending on the size and natural habitats [36, 40, 43, 44, 46]. They change their behavior according to temperature changes. In the months of May–October, the moths can be found in Belgium and Netherlands. The male is smaller than the female and has a scalloped upper edge of the wings. Male moth is 10–15 mm in length with beige color and light/dark markings (Figure 8). Male moth can use ultrasound from these scallops to find mating. Female moth is 15–20 mm in length, which is larger than the male wingspans. Their wingspans look like clothes folded at a shallow angle over the body. The wings have scales darker than males with a dirty brown/gray color, and occasionally with a minor bronze tinge [36, 40, 43, 44, 53]. Female moth has a sex pheromone called “Nonanal,” which can be found in wax and may explain how the wax moth finds an ideal place for their spawn. Mating of an adult occurs shortly by males and females, which can generate short ultrasonic signals. Males show the wings crawling to retrieve the female wings, which blow to release the pheromone, and lead to the first female approach. Later, the female will find a crevice to spawn. When a suitable place is found, the female pulls the body out to

Figure 2. Greater wax moth larvae (bottom), the last instar larvae (second), pupa (third), and cocoon (top)

penetrate the ovary into the crevice [36, 54–59]. They can lay about 2,000 eggs throughout their lives [40, 43, 47, 60].

The *G. mellonella* Immune System

G. mellonella has anatomical and physiological barriers to protecting them from microbes. This worm body or cuticle consists of a single mantle (epidermis), which is located on the base membrane. The cuticle is chitin-hardened to protect the insect from injury and infection. Similar to bronchial insects, chitin padding can be cured with age. In addition, inside of the trachea is not an appropriate and unfavorable condition for colonization and growth of microorganisms. Due to low humidity and less nutrients, when the infection passes

through the oral cavity, they will exhibit an intestinal structure to prevent infection. The structure of the stomach consists of the lining of chitin and intestinal conditions, such as pH and digestive enzymes, which are not favorable to intruder developers [61–65]. In addition, normal flora in their intestines plays an important role in reducing the amount of microorganisms that enter into the digestive tract with food. In turn, infections can penetrate physiological barriers, which will be defended by immune response system. Insect immunity only has a natural immune system that relies on the factors that encode pathogens in the recognition and infection process. However, they do not exhibit adaptive immune response, such as T-cell, B-cell, antibody, and immortal immune deficiency [66]. The innate immune system of insects is divided into two major parts: cellular immune response and humoral immune response (Figures 3 and 4) [67–69].

Cellular immune response of the moth is mediated by hemocytes, which are phagocytic cells. The hemocytes can be found in insect blood (hemolymph). Hemocytes are not only responsible for engulf intruders in the phagocytosis process, but also play a significant role in encapsulation and clotting by capturing the microorganisms in multicellular structures called nodules or capsules (Figure 3)

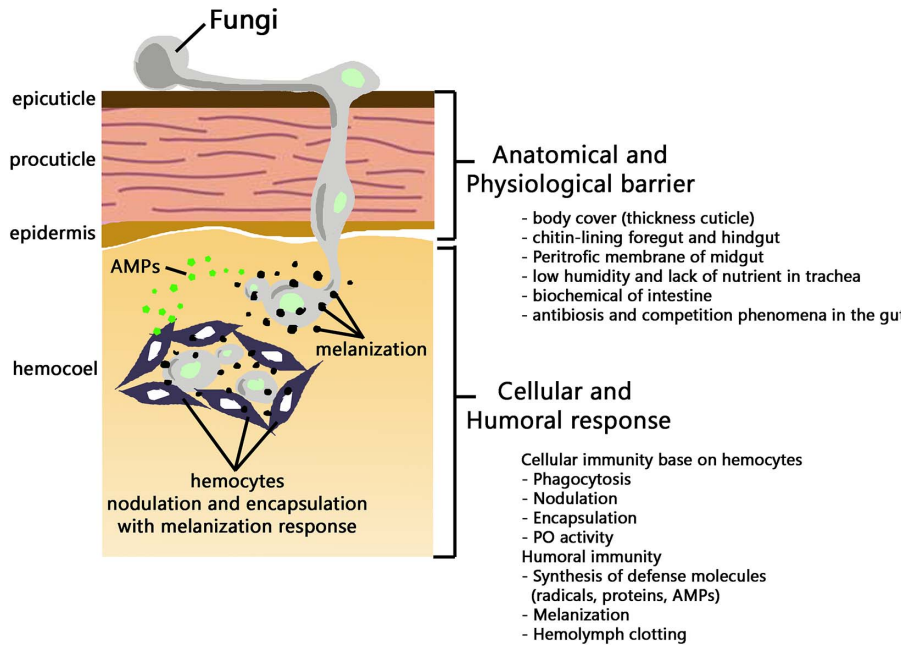

Figure 3. Immunity of *G. mellonella*

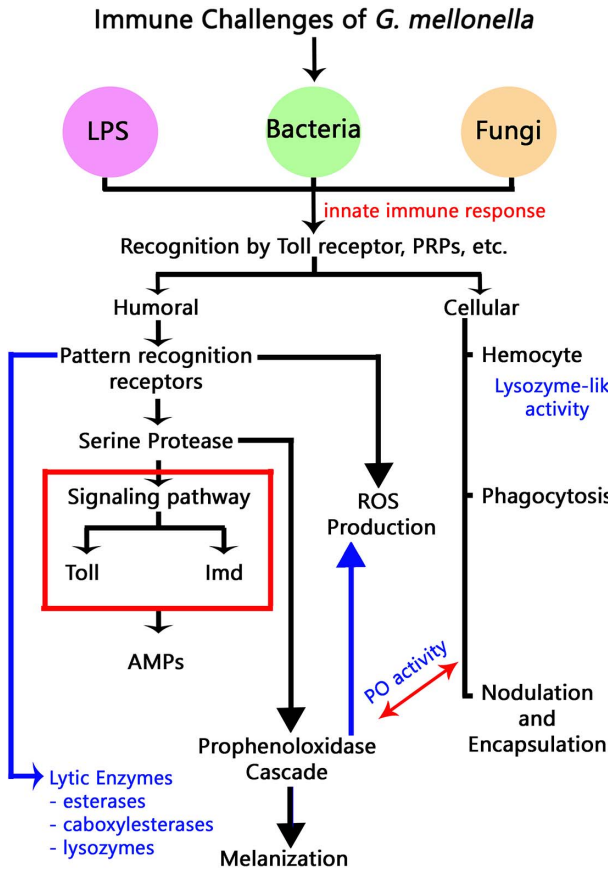

Figure 4. A schematic representation of larvae protected by anatomical and physiological barriers and by cellular and humoral reactions

[9, 68, 69]. Insects can produce several types of hemocytes that can be traditionally identified using morphological, histochemical, and functional characteristics [70, 71]. Six out of eight types of hemocytes in insects have been identified in *G. mellonella*, such as prohemocytes, plasmatocytes, granular cells, coagulocytes, spherulocytes, and oenocytoids (Table I) [9, 72]. In *G. mellonella*, plasmatocytes and granular cells play a vital role in cell defense and involve in phagocytosis, nodule formation, and encapsulation. Plasmatocytes and granular cells are the most common hemocytes that act as the first barrier of immune cells. Plasmatocytes can be characterized by a leaf-like shape and produce lysosomal enzymes in their cytoplasm. Granular cells are smaller and contain many granules in the cytoplasm (Figure 3) [68, 69].

Table I. Components of *G. mellonella* innate immunity

Cellular response	Hemocytes	Prohemocytes	[9, 67–69, 72–74]
		Plasmatocytes	
		Granular cells	
		Coagulocytes	
		Spherulocytes	
		Oenocytoids	
Humoral response	Opsonins	Apolipophorin-III (apoL-III)	[75–77]
	Antimicrobial peptides (AMPs)	Lyzozymes	[78, 80–84]
		Cecropin	
		Morcin-like peptides	
		Gloverin	
		Galiomycin	
		Gallerimycin	
		Galleria defensin	
		Gm proline-rich peptides 1 and 2	
		Gm anionic peptides 1 and 2	
		Inducible serine protease inhibitor 2	
		Heliocin-like peptides	
		x-tox	
		Gm apolipophorin	
	Melanization	Phenoloxidase pathway	[85, 86]

Immunological function of *G. mellonella* will be generated from the two cell types, which causes phagocytosis process, nodule formation, and encapsulation. First, penetration of microorganisms through the physiological barriers of insects can cause granular cells attack to the foreign target triggering the release of cytotoxic materials (e.g., polysaccharopeptide protein) and starting encapsulation process. These processes promote the attachment of multiple layers of plasmatocytes around the foreign materials resulting in a capsule formation. Encapsulation is mainly related to immune responses against larger microbes, such as protozoa and nematodes (including eggs and larvae). Moreover, phagocytosis is another process that plays an important role during immune response. Phagocytosis of insects has been believed to be similar to mammals involving with plasmatocytes and granular cells (Figure 3). Hemocytes of *G. mellonella* express proteins with high homology to calreticulin from human neutrophils, which involved in non-self recognition in cellular defense reactions. While phagocytosed, pathogens are killed by several mechanisms including reactive oxygen species, initiated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex and generated by the oxidation burst (Figure 4). In *G. mellonella,* hemocytes can produce p47 and p67 proteins, which can promote the production of superoxide. Similar to

human neutrophils, p47phox and p67phox proteins are translocated from cytosol to plasma membrane and can form NADPH complex [68].

Humoral responses are highly regulated by soluble effector molecules involving with defense molecules such as complement-like proteins, melanin, and antimicrobial peptides (AMPs). These interactions are responsible for immobilizing or killing the pathogens (Table I). Among them, reactive intermediates of oxygen, nitrogen, and AMPs exhibit antibacterial and/or antifungal properties (Figure 4) [69, 87–93]. Most of the defense peptides are produced in the body fat and presented in the hemolymph of *G. mellonella*. AMPs are found to be involved in destabilization of pathogen membranes by creating peptide or lipid-lined pores. In addition, AMPs may differently interfere with the cellular membranes suggesting that some defense molecules can enter the cell and interfere with the physiological processes, such as replication, transcription, and translation (Figure 4). Moreover, modes of defense peptide action can be found in the many review articles [94–96]. It can be concluded that there are four main features of AMPs. First, they are selective toxicities, which can act differently against infecting microorganisms without disturbing the body of the host. Second, their actions are shorter than the doubling time of infection. Third, the AMPs possess broad spectrum of activity. Finally, they do not develop bacterial resistance (Figure 4) [97]. Furthermore, the humoral system also includes melanization process, which is complex enzymatic cascade. Melanin is mostly synthesized during coagulation process, nodule formation, or capsule formation at the injury site, wound, and pathogen encapsulation areas (Figures 5 and 6) [68, 85, 98–101].

Many humoral factors regulate the hemocytes function and vice versa. Hemocytes synthesize and secrete humoral molecules to the hemolymph, such as defense peptides and stress proteins [68, 102]. Pathogens also develop themselves to pass the insect immune systems using their strategies to penetrate anatomical and physiological barriers. They synthesize or secrete many biological compounds, such as enzymes digesting host tissues. In addition, wound or injury on insect cuticle can be represented as the gate of infection for microorganisms. Pathogens avoid immunological recognition from insect immune mechanisms. They try to hide the immune elicitors (pathogen-associated molecular pattern) by changing the composition and structure of cell wall, causing colonization where the hemocytes cannot reach [69, 103]. Finally, they secrete virulence factors to inhibit activity of insect defense molecules. Proteases are the most enzymes that pathogen release to digest insect hemolymph and AMPs. Nevertheless, this virulence factors can be secreted from pathogens, which can stimulate insect immune response [104, 105].

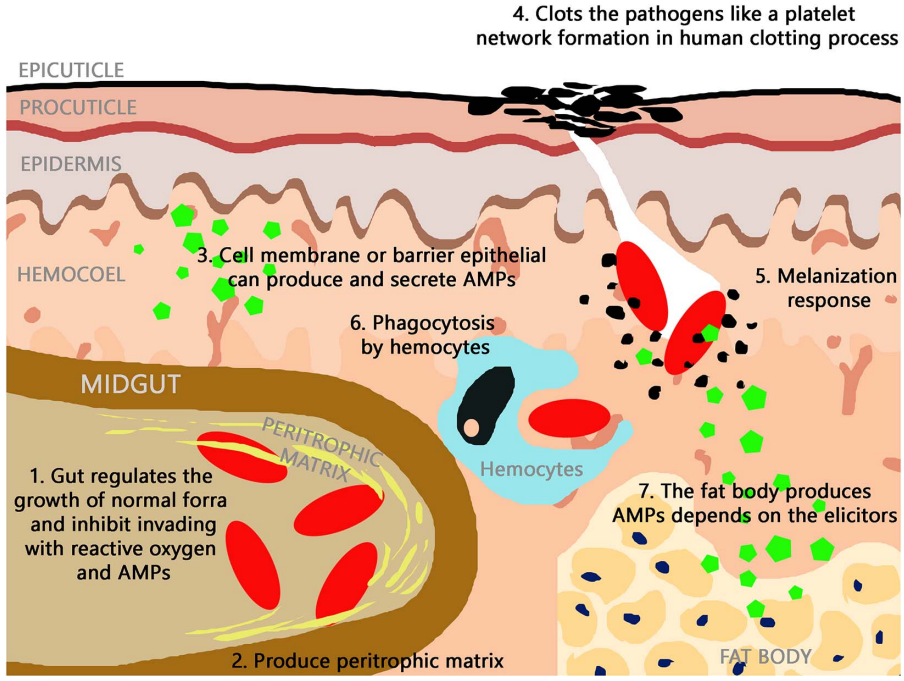

Figure 5. The cellular and humoral immune and melanization responses in *G. mellonella*

Figure 6. Spot/tail lines on *G. mellonella* cuticles. Melanization comprises the synthesis and deposition of melanin to encapsulate pathogens at the wound site followed by hemolymph coagulation and opsonization typically

Benefits of *G. mellonella*

It is widely accepted that *G. mellonella* is an enemy of bees. However, previous studies have found that this worm also plays an important role in agricultural and industry [106–108]. At present, the breeding of *G. mellonella* in commercial scale is widely used for agriculture industries. Studies have shown that storage of the worms at a cool temperature can prolong the survival time without eating. Moreover, microbes in worm guts have been demonstrated to degrade polyethylene, which will be beneficial for plastic disposal process. *G. mellonella* may find wide applications in commercial culture, that is it can rear on a mixture of cereal grain, bran, honey, and glycerol, which is helpful in agriculture. Therefore, *G. mellonella* may exhibit potential benefits to use as food for exotic pets. In addition, studies in the field of *G. mellonella* have been found active at present.

Developing *G. mellonella* as an Alternative Model in Animal Research

At present, the incidence of opportunistic infections in the bloodstream of patients has been increased rapidly, especially in immunocompromised patients. In addition, they are many new species of pathogens from antibiotic resistance that are often infected from the intensive care unit [109–111]. Within these problems, medical professions and scientists are trying to elucidating the mechanisms of infections. In order to address these questions, murine models are often used.

Murine model is one of the commonly used laboratory animal models with regard to pathogenic infection as the model exhibits similar physiological and immune systems to human. However, murine models are still having many limitations concerning ethical and logistical issues. In addition, long lifespan of murine animals may provide a contrary evaluation when compared to the period of pathogens evolution. Therefore, experimentation with an alternative model of invertebrates will provide numerous advantages than murine models. Although invertebrates are evolutionarily separated from mammals several thousand years ago, but they exhibit the innate immune system that is similar to mammals [4]. Moreover, invertebrate models exhibit low cost of maintenance, feasibility, and short lifespan suitable for evaluating in large population. The current limitations on the use of invertebrate animals are still relatively low, as models are simple and convenient. In Thailand, four invertebrates are being used and the most popular species is *G. mellonella* or greater wax moth.

The larvae of *G. mellonella* have been first used to study entomophatogenic fungi. The study has demonstrated that *G. mellonella* larva is a good model for studying human pathogens. In 2000, Cotter et al. [112] have shown that *G. mellonella* larvae can be killed by many bacterial and fungal human pathogens. However, the larvae can survive when culturing with non-pathogen organisms, which make them suitable for studying the infectious diseases. At present, *G. mellonella* is increasingly used as an animal model for studying the virulence factors of bacterial and fungal pathogens, insect immune response, toxicology, and disease pathogenesis (Figure 7). This model can be used as an alternative model for small mammals such as mice or rabbits in scientific experiments. These worms have been proven as a good model for studying innate immune system. In addition, it has both cellular and humoral immune responses but there is no adaptive immune response that makes to avoid the interference factors. In genetics, they can be used to study inherited sterility.

Moreover, *G. mellonella* larvae can be maintained at temperature ranging between 15 and 37 °C, which can survive well at human body temperature [113]. Thus, the model is well suited to study human pathogens as the pathogenicity of organisms, as virulence factors are known to be regulated by temperature. The convenient size of *G. mellonella* larvae is 2–3 cm in length, which is easy to work with and large enough to allow straightforward handling with accurate dosing. In addition, the worms do not require any specific tools, which also reduce the experimental cost when compared with small mammals (usually mice, hamsters, or guinea pigs).

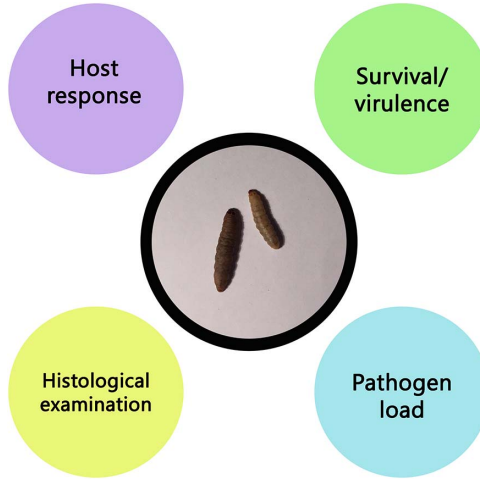

Figure 7. The usage of *G. mellonella* as animal models

G. mellonella larvae are widely used to study human pathogens, especially bacteria such as *Staphylococcus aureus*, *Proteus vulgaris*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, and *Enterococcus faecalis* [114–119]. The *G. mellonella* larvae are also widely used to study fungal infection such as *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Candida albicans* [15, 19, 120–123]. At present, *G. mellonella* has been used to screen bacterial and fungal strains and identify genes involved in disease pathogenesis or therapeutic compounds. In a subsequent study, it has been proven that the model is particularly useful in identifying the chemical compounds absorption with favorable bioavailability.

Rearing *G. mellonella* to be Used as an Animal Model in Research

Rearing methods of wax moth have been differently developed to use as a supplement for reptiles, birds, fish, and small mammals or used as animal models in scientific research. Thus, there are many methods in the scientific literature, making it difficult to determine a standard rearing method. Nevertheless, most rearing methods are very similar. Males and females should be placed in containers with diet [124]. Moreover, the culture environment should be adjusted to an appropriate temperature. Eggs can develop quickly at 29–35 °C and slowly at less than 18 °C. Moths are preferred to lay eggs on the surface that can protect their eggs such as cracks and crevices. Therefore, many rearing methods recommend using paper clips held by the fold-layered paper or wax paper. In the larval container, the wax paper or corrugated cardboard can be added after the larvae begin to spin cocoons [36].

Warren and Huddleston [47] have studied the effect of humidity and temperature on various life stages of *G. mellonella*. The results have shown that wax moths are nocturnal insects that thrive in dark, warm, and poorly ventilated areas like beehive. Therefore, the most favorable environment for rearing the wax moths is approximately 30 °C, 70% relative humidity, and darkness.

The size of containers depends on the number of wax moth or purpose of use such as larval chamber, mating chamber, or oviposition chamber. Marston et al. [48] have proposed a large mass-rearing program that separated multiple rooms with diets prepared by a cement mixer and use sieve to collect eggs. A study by Waterhouse [125] has demonstrated the use of paper clip to seal the plastic. A study by Haydak [126] has demonstrated the use mason jars covered with mesh. A study by Bronskill [127] has demonstrated the use of lantern-globe cage that has been invented to replace the mason jars. From many

studies, it can be concluded that metal, glass, or plastic can be used, but wood, cardboard, and paperboard should be avoided because the larvae can chew through them.

The Experimental Method Using Larvae of *G. mellonella* as Infection Models (Modified from Fuchs et al. [17])

Comparative studies between the *G. mellonella* model and murine model have been shown to exhibit the similar results. By comparison, pathogenic infection in *G. mellonella* is simpler than mammal model and requires less training. This model does not require the specialized housing and the lethal infection time is faster than mammals' model. At present, the larvae of *G. mellonella* have been widely used to study important human pathogens, including Gram-positive and Gram-negative bacteria, several pathogenic fungi, and some viruses [114–121, 128–135]. The last instar larvae can be developed from eggs about 5 weeks, which can be experimented. These larvae stop feeding and start producing silk, which are 200–400 mg or 2–2.5 cm long in creamy color without gray-color marking on their cuticle. In each experiment condition, at least 10–20 larvae/group should be used (Figure 8). The larvae can be stored at 15 °C housed in petri dishes prior use and it is recommended that the larvae can be kept for 24 h to starve them before infection.

The selected larvae can be inoculated with pathogens via three methods, such as topical application, oral delivery, and injection. The topical method is decribed by pathogens absorption through larvae skin [136]. This method is modest, especially in terms of the effort required for inoculum on the larvae cuticle. The pathogens enter the larvae by penetrating into the cuticle. While facile is in execution, however, delivery method exhibits disadvantage as lacking the known number of infection. Therefore, we do not use topical application as typical method to infecting the larvae. In the oral delivery, the pathogens enter the larvae by feeding that has been reported as an infection method [137]. In this method, pathogens will be mixed with pollen in 1:1 ratio and placed in petri dishes to house the *G. mellonella* larvae. The pathogens enter the larva through ingestion, which can initiate the host's natural defense. However, the method is disadvantageous that infection doses are difficult to obtain. Therefore, we do not use oral route as a method of *G. mellonella* infection in our own laboratory. Both the oral and topical applications for the delivery methods have the same limitations found in *G. mellonella* host inoculated. In the most common infection route, intrahemocoelic injection through the last proleg has also been recommended (Figure 8). This delivery route by injection of a known

Figure 8. The larvae that are alive are creamy-colored with no spot on their cuticles (left). Larvae that are held between the fingers and needle is inserted at the site of the proleg to inoculum delivery (right)

number of pathogens dose to larvae hemocoel can be achieved. The first infection site is the last proleg. In addition, multiple injections such as in the case of drug delivery post-inoculation can rotate to the other last proleg. During the inoculation process, delivering the pathogens to the larvae hemocoel can also be performed using Hamilton syringe (Figure 8). The size of syringe and needle is important, in which the usage of 26-gauge needle and Hamilton syringe as the large syringe has been recommended or else needle can cause trauma leading to larvae death [17].

In research, larvae are inoculated with different pathogenic doses; thus, half-maximum lethal dose (LD_{50}) should be calculated. After infection, the larvae can be maintained at 25–37 °C. The study of microbial virulence in *G. mellonella* is typically assessed within 5–7 days. More recently, a health index scoring system has been introduced by evaluating the larvae health status according to four major observations: larvae mobility, cocoon formation, melanization, and survival. There are some visible differences in appearances of the larvae post-infection. They may appear black spot (spot/tail line) on cream-colored cuticle within an hour of infection due to a melanization process. Moreover, the larvae show low motility and completely death (black larvae). Moreover, plating larval extracts on agar plates or using bioluminescent microorganisms to detect the pathogen load by biophotonic imaging can be used to assess microbial virulence. These studies can also measure the microorganism proliferation inside the larvae during infection. In addition, pathology of the larvae can be observed in tissue section, as the retained pathogens can be stored in body fat and other internal structures after being injected into the hemocoel. Nevertheless, in research, the immune response during pathogenic infection also requires further studies in depth. *G. mellonella* immune response to pathogens can be observed by isolation hemocytes from larvae after infection.

Limitations in the Use of *G. mellonella* as a Model

Although *G. mellonella* is an excellent model for assessing the virulence of various microorganisms, it may not entirely represent biological information with regard to mammalian models. However, *G. mellonella* provides a promising strategy to quickly access and cost-effectively collect basic science information. However, one must consider that the *G. mellonella* infection still requires integrative knowledge and more pioneer studies. The model of *G. mellonella* may not wildly accept when compared to some other invertebrate models such as nematodes (*Caenorhabditis elegans*) or the fruit fly (*Drosophila melanogaster*) [16].

G. mellonella model also exhibits some limitations when studying their genetics. *G. mellonella* genome has not yet been fully sequenced, lack of available established methods to create mutant species, and lack of accessible data in microarrays or RNA interference libraries. Moreover, the most challenging in *G. mellonella* research is the lack of stock centers of *G. mellonella* under standard conditions. At present, we can purchase *G. mellonella* from commercial company, in which the larvae will be sold as pet food. Differences of genotypes and breeding conditions, such as housing temperature, light sources, and diet, can affect the results in laboratory experimentation [122, 138, 139]. Moreover, there are some differences in mortality rates after infection with pathogens among *G. mellonella* individuals.

G. mellonella as a Model Platform for Future Perspective

The several studies of *G. mellonella* as a model with human pathogen demonstrated the good results. Thus, this animal model might be the suitable model to basic medical knowledge especially the immunopathogenesis of pathogen, medical product development. However, this model has the limitations to strictly concerned, i.e., high level of light and heat sensitivity, which will be the confounder factors.

Conclusions

The invertebrate animal models are rapidly growing and attractive for many researches. However, the *G. mellonella* model cannot replace the mammalian model completely. Instead, they should serve as an additional, simple to use, low cost, short life span, and rapid screening method to expand our knowledge.

Finally, there are no ethical constraints in the use of invertebrates, which further facilitates their use for *in vivo* experimentation. Recently, *G. mellonella* has become increasingly popular for *in vivo* research. However, the immune response of this model is still unclear and lack of the reference strains provided under standard conditions to support comparative experiments conducted by different research groups and the genomic information are also limited. Furthermore, the experimental conditions often vary between individual researches and require standard to reduce ambiguity.

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Conflict of Interest

The authors report no conflict of interest. They alone are responsible for the content and the writing of the paper.

Ethics

All applicable international, national, and/or instutional guildlines for the care and use of animals were followed with ethical approved certificate number MU-IACUC 2018/015, Mahidol University, Thailand.

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