Locusts: A Model to Investigate Human Disease and Sickness Behavior

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ABSTRACT: Traditionally, vertebrate models have been utilized and are viewed as more pertinent; however, we propose the application of an invertebrate model such as locusts to study human disease and sickness behavior at an early phase of research. This model has numerous benefits, namely, expense, swiftness, procedural convenience, and ethical acceptance. For example, the injection of immunogen-induced anorexia behavior in rats and locusts *in vivo* are analogous. Moreover, the presence of a brain barrier in locusts reveals remarkable similarities in molecular methods utilized by *E. coli* K1 to traverse the central nervous system of rats and locusts, consequently providing a worthwhile model to investigate pathogenesis. The presence of cytokines in these insects and presence of a brain barrier (which is physiologically relevant to human blood–brain barrier) makes it a relevant model in determining disease mechanisms and invasion of the brain by central nervous system pathogens.

KEYWORDS: *Escherichia coli*, sickness behavior, pathogenicity, laminarin, anorexia, blood–brain barrier, locusts

Here we propose the use of nonvertebrate *in vivo* models to study human diseases associated with neurodegenerative conditions and microbial infections. Experimentation on vertebrates has drawn attention due to ethical apprehensions and this has driven research into alternative nonvertebrate and nonmammalian models. Moreover, these alternative model systems also offer the added advantages of speed and lower expenses, less infrastructure requirements, and legislative adherence. In this regard, the utilization of insects such as locusts to understand infection of the brain and sickness behavior can be of value at an early stage of research to identify disease mechanisms, applicable to humans.

In particular, sickness behavior that may comprise of physiological and behavioral variations that manifest in infected animals, including fever and anorexia, can be modeled in locusts. Similar to mammals, sickness behavior in insects is cytokine-mediated; however, only limited insect cytokines are recognized. Moreover, the locust immune system comprises humoral-based and cell-mediated immune reactions that include synergy of hemocytes, fat bodies, and circulating hemolymph peptides for containment or deactivation of attacking pathogens. For such a harmonized response, locust cytokines are fundamental in mediation of immune reactions.¹ Previous work has described the phenomena of immunogen-induced anorexia in locusts, drawing parallels with sickness-induced anorexia in mammals. This was demonstrated using feeding assays using adult locusts (15–25 days old) at 30–35 °C. In this assay, each animal was observed consuming wheat seedlings and then deprived of their diet for 2 h. Following this period of enforced fasting, the insects were injected with 20 μ L of either Ringer solution or laminarin (10 μ g in 20 μ L of Ringer), and β -1,3 glucan immunogen. Individual insects were then placed on fresh wheat seedlings, so insects are prepared to feed when presented with wheat seedlings. Locusts can be

observed individually for their feeding activity. In some experiments, a serotonin receptor blocker, mianserin, was injected 10 min prior to laminarin, and in other investigations, serotonin alone was injected 10 min before observing feeding behavior. The locusts depicted prominent anorexia when their immune system was primed by laminarin injection; however, whether or not cytokines participate in locust anorexia remains to be elucidated. The majority of locusts fed after receiving laminarin (10 μ g) given 10 min following injection with mianserin, whereas locusts inoculated with serotonin alone revealed a greater latency to feed but with no effect on the meal duration. Injection with octopamine revealed no obvious effects on their feeding behavior. Notably, Mancilla-Diaz et al.² observed similar results on feeding behavior in rats. Taken together, this data suggests that the use of locusts is a relevant model to investigate sickness behavior.

The presence of brain barrier (physiologically relevant to human blood–brain barrier) makes it a relevant model in determining pathogenicity and invasion of the brain by CNS pathogens. The vertebrate blood–brain barrier is comprised of a distinct cell layer connected by tight junctions. These tight junctions consist of three key fundamental membrane proteins (namely, occludin, claudin, and the junction adhesion molecule) and the cytoplasmic proteins (zonula-1, ZO-2, and ZO-3). There are also adherens junctions (constituted of cadherin membrane protein) that fuse with catenin and are

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associated with the actin cytoskeleton, establishing adhesive connexions among cells. Moreover, zonula or accessory proteins deliver structural assistance and bind to the tight junction integral membrane proteins, thus linking the membrane proteins to the actin cytoskeleton and causing it to be extremely selective. Insects like locusts exhibit a circulatory system that is open, with the hemolymph immersing all tissues; however, the peripheral nervous system and the brain are separated and protected with a barrier. The insect brain barrier is also contingent on presence of tight junctions among glial and/or perineurial cells; for example, in *Drosophila*, the molecular constituents of tight junctions are comparable with the tight junctions of vertebrates.^{3–5} The insect brain barrier and ion selectivity have been well-studied. However, there is limited data on barrier resistance to penetration by pathogens. It is evident that there are analogies between the processes by which *E. coli* K1 traverses the CNS of mammals^{6,7} and of locusts, with reference to the molecular details. For example, when the locust abdomen or hemocoel (insertion of needle was made precisely between the intersegmental membrane between two terga of the abdomen) was injected with neuropathogenic *E. coli* K1 (2×10^6 CFU suspended in 20 μ L of LB broth), bacteria were retrieved from the dissected brains within 24 h. Furthermore, OmpA was observed to participate in host cell recognition, and CNF-1, in stimulating phagocytosis of bacteria, depicted noteworthy similarities between bacterial invasion of the locust brain and their ability to colonize human endothelial cells constituting the blood–brain barrier.

In summary, a whole-organism methodology is indispensable to understand the physiology of disease and in acquiring further comprehension of host–pathogen interactions. Although mammalian models are instantaneously more pertinent, the locust model depicted herein is a constructive tool to distinguish molecules participating in both aspects of host–bacterial interactions. We envisage that the locust model will be useful to yield prospective leads that can be successively investigated in mammalian systems, thus narrowing down the search of such molecules and reducing the use of mammalian models. Furthermore, the use of invertebrates to study molecular mechanisms of relevant human diseases and sickness behavior is an apt response to the communicated aspirations of governments and members of the public in minimizing and substituting for the use of animals in research, particularly of mammals. Successful use of the locust model could minimize the numbers of mammals utilized overall by scientists. Furthermore, as insects rely on their innate immune system for protection against infection, utilization of an insect model is especially applicable in the comprehension of neonatal meningitis caused by *E. coli* K1, the regulation of which has substantial dependency on the innate immune systems. Even though vertebrate models are viewed as more physiologically relevant, our proposal is the utilization of the invertebrate locust model at an early phase that presents many advantages because of lower expenses, procedural convenience, and ethical tolerance as well as speed.

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All authors contributed equally to the manuscript and will act as guarantors.

Notes

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REFERENCES

- (1) Duressa, T. F., Boonen, K., Hayakawa, Y., and Huybrechts, R. (2015) Identification and functional characterization of a novel locust peptide belonging to the family of insect growth blocking peptides. *Peptides* 74, 23–32.
- (2) Mancilla-Diaz, J. M., Escartin-Perez, R. E., Lopez-Alonso, V. E., and Cruz-Morales, S. E. (2002) Effect of 5-HT in mianserin-pretreated rats on the structure of feeding behavior. *Eur. Neuro-psychopharmacol.* 12, 445–451.
- (3) Siddiqui, R., Edwards-Smallbone, J., Flynn, r., and Khan, N. A. (2012) Next generation of non-mammalian blood-brain barrier models to study parasitic infections of the central nervous system. *Virulence* 3, 159–163.
- (4) Siddiqui, R., Pleass, R., Mortazavi, P., and Khan, N. A. (2011) Non-vertebrate models to study parasite invasion of the central nervous system. *Trends Parasitol.* 27, 5–10.
- (5) Daneman, R., and Barres, B. A. (2005) The blood-brain barrier—lessons from moody flies. *Cell* 123, 9–12.
- (6) Khan, N. A., and Goldsworthy, G. J. (2007) Novel model to study virulence determinants of *Escherichia coli* K1. *Infect. Immun.* 75, 5735–5739.
- (7) Mokri-Moayyed, B., Goldsworthy, G. J., and Khan, N. A. (2008) Development of a novel *ex vivo* insect model for studying virulence determinants of *Escherichia coli* K1. *J. Med. Microbiol.* 57, 106–110.