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Mosquito Immunobiology: The Intersection of Vector Health and Vector Competence

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Abstract

As holometabolous insects that occupy distinct aquatic and terrestrial environments in larval and adult stages and utilize hematophagy for nutrient acquisition, mosquitoes are subjected to a wide variety of symbiotic interactions. Indeed, mosquitoes play host to endosymbiotic, entomopathogenic, and mosquito-borne organisms, including protozoa, viruses, bacteria, fungi, fungal-like organisms, and metazoans, all of which trigger and shape innate infection-response capacity. Depending on the infection or interaction, the mosquito may employ, for example, cellular and humoral immune effectors for septic infections in the hemocoel, humoral infection responses in the midgut lumen, and RNA interference and programmed cell death for intracellular pathogens. These responses often function in concert, regardless of the infection type, and provide a robust front to combat infection. Mosquito-borne pathogens and entomopathogens overcome these immune responses, employing avoidance or suppression strategies. Burgeoning methodologies are capitalizing on this concerted deployment of immune responses to control mosquito-borne disease.

1. INTRODUCTION

Mosquito immunity provides powerful protection in a range of infections wherein mosquitoes are victims in entomopathogenic infections, the unwitting vector for mosquito-borne pathogens, or the symbiotic other for communities of commensal organisms. In the face of so many challenges to the mosquito immune system, there is an elaborate network of recognition, signaling, signal modification, and effector pathways elicited and deployed. There has been tremendous progress on molecular and biochemical characterization of these immune players, particularly with genome sequence data for major mosquito vector species, including 11 *Anopheles* species that are major malaria vectors as well as *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* (12, 48, 124, 168). Comparative genome analyses reveal contractions and expansions in canonical immunity genes; the *Cx. quinquefasciatus* genome encodes 500 such genes, as compared to 417 and 380 in *Ae. aegypti* and *Anopheles gambiae*, respectively (12).

The expansion and diversity of immunity and infection response genes are not functions of one-to-one adaptation in gene families or pathways to specific pathogens; the mosquito immune response lacks specificity. For example, the Toll pathway serves a dual function in embryonic development and innate immunity. In mosquitoes, the Toll pathway is elicited by and important for the response to entomopathogenic fungi, Gram-positive and Gram-negative bacteria, *Plasmodium* parasites, dengue virus (DENV), and *Wolbachia* endosymbionts (60, 70, 133, 153). Distinct immune pathways and effectors then are used in the context of various infections and also are deployed simultaneously. During a septic infection of *Ae. aegypti*, phenoloxidase (a hallmark of melanization) and defensin (an antimicrobial peptide) colocalize on bacterial cells (85).

From these examples, it appears that there is strength and efficiency in deploying immune response pathways and effectors that are co-opted for use in multiple physiologic functions and in being able to bring to bear the full arsenal of responses on an infection. The demands on the immune response become particularly clear when considering the spectrum of symbiotic, entomopathogenic, and mosquito-borne pathogens.

2. CONTEXTUALIZING IMMUNITY IN MOSQUITO HOSTS

2.1. Mosquitoes Are Symbiotic Others for Whole Communities of Commensal Organisms

Like all animals and plants, mosquitoes are holobionts, consisting of a complex community of organisms. Deep sequencing technology is revealing new components of the holobiont, particularly bacteria and viruses, at an exciting pace. The following section provides a snapshot of mosquito commensals.

2.1.1. Protozoa. Some notable examples of protozoa are apicomplexan gregarines and trypanosomatid parasites in the genus *Crithidia*, which generally are considered nonpathogenic (40). That said, these are frequently associated with mosquitoes. *Ascogregarina barretti*, a gregarine that infects *Ae. triseriatus*, can reach prevalence of up to 100% in field-caught mosquitoes (138).

2.1.2. Bacteria. The mosquito midgut is populated with bacteria that are of increasing interest for their role in shaping the immune response. These communities change and shift with mosquito life stage and nutritional status, geography, and phenology. For example, the mean number of culturable bacteria in larval stages of field-collected *Ae. triseriatus* and *Cx. pipiens* exceeded 36,000 per midgut. From the same populations, newly emerged adults had mean counts of 141 and

32 bacteria, respectively (56). In *An. coluzzii* and *An. gambiae* collected in Ghana, there is evidence of carryover of bacterial species constituents in adults from larvae, and the diversity of species populating the guts of these species is more variable during the dry season (4). Eclosion and disposition of the meconium reduce the number of bacteria retained in adult mosquitoes, but this changes dramatically with blood feeding. Female *Ae. aeygpti* midguts are "almost totally occupied by bacteria" as visualized by scanning electron microscopy at 48 h postfeeding (77, p. 275).

2.1.3. Viruses. Mosquitoes are host to many new viruses that defied detection and description prior to the advent of high-throughput sequencing technology, because they do not cause overt pathology in, and may only infect, mosquito hosts (29, 159). The insect-specific flaviviruses (ISFs) illustrate the symbiotic relationships between viruses and mosquitoes. These viruses depend on a strictly monoxenous life cycle based on vertical transmission and are not infectious to mammalian cells. Kamiti River virus was the first ISF, isolated from field-collected *Ae. macintoshi* larvae and pupae in Kenya (147); this provided initial evidence for vertical transmission. Studies with *Culex* flavivirus (CxFV) show that ISFs have extraordinarily high transovarial (100%) and filial infection rates (97%). CxFV infects numerous tissues in adult mosquitoes with no apparent pathology (146).

2.2. Victims of Entomopathogenic Infections

Aquatic habitats of mosquito larvae and pupae are rife with phyto- and zooplankta, including organisms that establish parasitic relationships with mosquitoes. The environment that an adult mosquito inhabits undoubtedly also puts it at risk for infection. A brief review of some representative examples of entomopathogens follows, with emphasis on life history and transmission strategies, pathobiology, and infection prevalence in field-caught mosquitoes.

2.2.1. Ciliates. Ciliates in the genus *Tetrahymena* are commonly found in larval breeding sites. For example, *Tetrahymena clarki* (syn. *Lambornella clarki*) was the most common parasite in *Ae. sierrensis* collected in California (167), and up to 8.5% of larvae were infected in breeding sites in Roraima, Brazil (9). Susceptibility and infection outcomes vary significantly depending on the host species; *T. pyriformis* is avirulent in *Ae. aegypti* but causes high mortality in *Cx. tarsalis* (76).

Cyst life stages of *Tetrahymena* species penetrate the cuticle of *Ae. sierrensis* larvae (**Figure 1**). Black melanized spots on the cuticle mark the locations of invasion events, and the invading cells cause septic ciliatosis (49). In cases where the infection persists to the adult life stage, the parasites cause sterilization (167).

2.2.2. Fungi and fungal-like organisms. Larval and adult mosquitoes encounter a broad spectrum of entomopathogenic fungi and fungal-like organisms (148). Some of these, for which associated pathology has been described, are showcased here.

Microsporidia in several genera infect juvenile and adult mosquitoes. *Brachiola algerae* and *Vavraia culicis* exhibit a broad host range and limited tissue specificity. These species infect mosquito larvae only horizontally and per os. Microsporidia with more complex life cycles exploit a copepod intermediate host. Others use horizontal, transstadial, and vertical transmission (7, 17). The pathobiology induced by the infection depends on the parasite's developmental trajectory. Larval *Ae. cantor* are infected by feeding on *Amblyospora connecticus* spores in the water (**Figure 1**); the spores germinate, invade the midgut epithelium, and spread to muscles and oenocytes. This infection process does not kill the larva, and the parasite proceeds to be transmitted transstadially to adult mosquitoes. When infected female mosquitoes take a blood meal, *A. connecticus* spores invade the nurse cells and attain transovarial transmission. The resulting female F1s can perpetuate

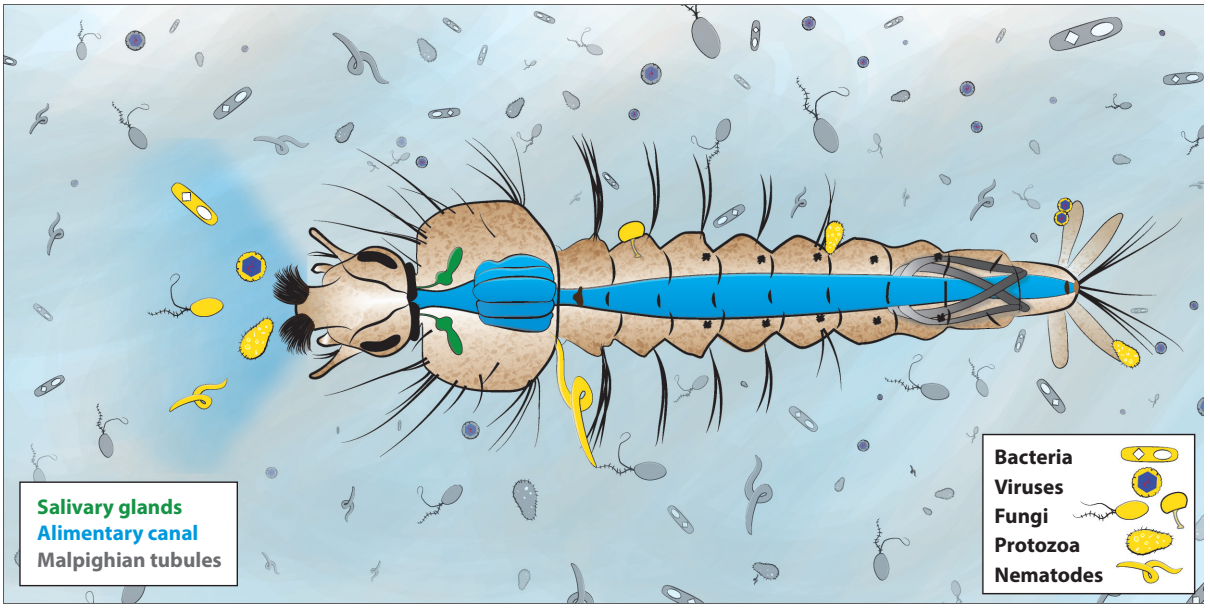

Figure 1

The infectious space for mosquito larvae. Mosquito larvae, represented by this fourth-instar *Anopheles gambiae*, face a number of potential pathogens in their aqueous environment. Presumably, the majority of infections occur through the oral route, including infections with opportunistic bacterial and fungal pathogens, such as *Bacillus thuringiensis*, *B. sphaericus*, and several species of microsporidia, as well as pathogenic baculoviruses in the Nucleopolyhedrovirus group. However, a few pathogens enter their larval host through the cuticle, including several nematode species in the family of Mermithidae, which enter the hemocoel by direct penetration especially at the thoracic to abdomen border. In addition, fungi such as *Beauveria bassiana* or *Metarhizium anisopliae* and certain ciliates, including *Lambornella clarki*, may penetrate the cuticle directly. Finally, anal papillae serve as the entry site for certain viruses, including densovirus, and possibly mosquito iridescent virus as well as ciliates. For references and further information, please see Section 2. Image credit: Victoria S. Rhodes.

this life cycle. However, in some female and all male F1s, the parasites destroy fat body tissues, causing significant mortality. Dead F1 larvae release infectious *A. connecticus* microsporidia that are consumed by copepods, in which the infection is also lethal, and so the cycle continues. In the field, the prevalence of infection with these parasites in *Ae. cantor* can exceed 90% (7).

The oomycete *Lagenidium giganteum* exemplifies the potential pathogenic infection of a watermold. Zoospores in the water column encounter the cuticle of a mosquito larva, and molecular signatures from chitin trigger encystment. Germination and host invasion proceed, and mycelium filaments consume and completely replace mosquito tissue, leaving only the cuticle; postmortem, mycelia produce asexual fruiting structures containing zoospores that will initiate new infections (98).

Coelomomyces are true fungi that infect aquatic larvae of Diptera in a heteroxenous life cycle that involves copepod intermediate hosts (reviewed in References 65 and 148). *Coelomomyces stegomyiae* illustrates the life cycle and pathology of these fungi. Planogametes are released in the water as a result of lethal infection of a copepod host. A biflagellate zygote encysts on the intersegmental cuticle and produces a penetration tube that enters the hemocoel (148). Fungal hyphae penetrate the musculature, hematopoetic organ, gut, Malpighian tubules, and fat body (154). Fourth instars succumb to the infection and release meiozoospores that are infectious to the intermediate host.

Beauveria bassiana infects a broad range of insects, including mosquito larvae and adults. In the original literature, larval-stage mosquitoes were exposed to conidia on the water surface. Infections initiate when conidia contact perispiracular lobes at the apex of the siphon. The conidia germinate and send germ tubes into the cuticle. Clark et al. (50) noted "numerous melanized hyphae" shortly after infection. By four days postexposure, the siphon fills with mycelia, and hyphae were evident in the hemocoel. Clark and colleagues speculated that death resulted from hyphae blocking the trachea and inducing suffocation, or by some type of toxin. *B. bassiana* infection also kills adults in a number of mosquito species (26). Infection with seven different strains of *B. bassiana* in *An. stephensi* produces a range of mortality phenotypes, with a most virulent strain that induces high mortality (80% in six days), and sublethal effects that include infected mosquitoes being significantly less likely to take a blood meal after exposure (27, 38).

2.2.3. Viruses.

2.2.3.1. DNA viruses. The baculoviruses that infect mosquitoes form large inclusion bodies of replicating virions in midgut epithelial cells in the gastric cecae and posterior stomach (108). Infections are initiated with ingestion of the occluded virus (see **Figure 1**), which moves through the peritrophic matrix to infect the midgut epithelium. The virus replicates and invades new cells in budded virus form. Within days, the infection produces new occlusion bodies that appear as white cysts, and destruction of the gut proves lethal to the majority of infected larvae. In some instances, survivors can maintain the infection transstadially and the occlusion bodies are passed with the meconium, which could inoculate new aquatic habitats with infectious virions (16). As an example of the epizootic potential of the baculoviruses, *Culex nigripalpus* nucleopolyhedrosis virus infection prevalence reached up to 60% in *Cx. nigripalpus* collected in Florida (15).

2.2.3.2. Iridoviruses. The mosquito iridoviruses are large icosahedral dsDNA viruses that infect fat body cells and produce iridescent coloration. Regular mosquito iridescent virus infects *Ae. taeniorhyncus* and causes larvae to turn yellow or yellow-green, sluggish, and moribund. Infection is most pathogenic to mosquitoes that acquire the virus in early instars. Mosquitoes that are exposed as third or fourth instars have increased chances of surviving infection and carrying the infection transstadially to adulthood. Transovarial (as opposed to transovum) transmission occurs at a very high rate (up to 100%) (107).

2.2.3.3. Densoviruses. The densoviruses exhibit both horizontal and vertical transmission. The infection begins with densoviruses infecting larvae via the anal papillae (**Figure 1**); indeed, tissue tropisms include the fat body, neurons, and hemocytes but not midgut epithelium. Densovirus infection is most pathogenic to early instars, in a dose-dependent manner, such that up to 92% of two-day-old larvae are infected, infection disseminates in up to 60% of individuals, and 70% die as a result of infection. The anal papillae of many *Aedes aegypti* densovirus–infected mosquitoes are lost, undergo shrinkage, or show signs of melanization (165). Transstadial transmission is evident in larvae that survive infection, from which vertical and horizontal sexual transmission occurs. For example, an *Aedes aegypti* densovirus (Thailand), AThDNV, is pathogenic for several *Aedes* species and readily infects *Ae. aegypti* and *Ae. albopictus*. AThDNV infection in *Ae. aegypti* induces up to 51% mortality as compared to 82% in *Ae. albopictus*. In surviving larvae, transstadial and then very efficient vertical transmission were observed—58% of F1s were infected, and the virus was maintained for six generations (102). In the field, collections from 11 provinces throughout Thailand revealed 44% prevalence in adult *Ae. aegypti* but no natural infections of *Ae. albopictus* (102).

2.2.4. Bacteria. Two of the best known entompathogenic bacteria are in widespread commercial use for control of mosquito larvae—*Bacillus thuringiensis* var. *israelensis* (Bti) and *Lysinibacillus spaericus*. Indeed, these biopesticides are in such widespread use that there is evidence of resistance in the field (175). During spore formation, these bacteria produce crystalline toxins that are highly pathogenic to gut cells. In *Ae. aegypti* larvae, ingested Bti spores release toxins that interact with the midgut epithelial villi and induce an enlargement of intra- and intercellular spaces in the cell. Endoplasmic reticula disintegrate, mitochondria lose the internal cristae, microvilli disappear, and the peritrophic matrix is malformed (41). These early observations are explained by toxin activation and activity, receptor binding, and pore formation, which have been the subject of intense research efforts and were recently reviewed in Reference 175.

2.2.5. Nematodes. Nematode species, particularly mermithids, are cosmopolitan in distribution and infect many species in multiple genera, often with a high prevalence. Parasites in a prelarva stage penetrate the cuticle of a larval mosquito (**Figure 1**), enter the hemocoel, and grow to a postparasitic stage that emerges from the fourth instar, resulting in death of the host (137). For example, *Romanomermis iyengari* is infectious and lethal to ten mosquito species in five genera, and the infection was most pathogenic if acquired during the second versus fourth instar (131).

2.3. Unwitting Vector Hosts

Mosquito-borne pathogens (MBPs) enter the mosquito body with the blood meal (**Figure 2**). Like the entomopathogens, MBPs have to navigate to and infect various tissue types to achieve transmission. By necessity, the MBPs cause little pathology in the mosquito host, yet vector–MBP interactions have been the focus of the vast majority of mechanistic work on infection responses in mosquitoes.

2.3.1. Viruses. The mosquito-borne arboviruses fall mainly into the Flaviviridae, Togaviridae, and Bunyaviridae families. By definition, these viruses are heteroxenous and pass from a vertebrate host to a vector horizontally. In the generalized infection scheme, arboviruses enter the body with a viremic blood meal (**Figure 2**) and invade and infect midgut epithelial cells. The infection rapidly disseminates from small foci to much of the midgut tissue (see, for example, Reference 67) or sometimes progresses more rapidly, as does Chikungunya virus (CHIKV), which can reach the salivary glands within two days of infection (58). From the midgut, viruses escape the basal lamina and disseminate to the hemocoel (69). It is possible that free virions transit to the salivary glands, but hemocytes are receptive to arbovirus infection and replication (134). Viruses invade salivary gland epithelial cells and again replicate. This process generally causes little overt pathology. Exceptions to this rule include, for example, Eastern equine encephalitis virus infection in the midgut of *Culiseta inornata*, wherein infection produces corpse-like cells that are sloughed into the lumen (**Figure 3**) (170).

Some of the arboviruses also are transmitted vertically and transstadially. LaCrosse virus (LACV), a member of the Bunyaviridae, has a transovarial transmission (TOT) efficiency of over 50% (169). LACV infection in vertically infected larvae is evident in most tissues, and the virus is transstadially transmitted to produce infected male and female adults that also can pass the virus venereally (90). By contrast, transovarial transmission is much less efficient for the flaviviruses. A recent examination of TOT of Zika virus (ZIKV) in *Ae. aegypti* yielded no more than 3% infected F1s (156).

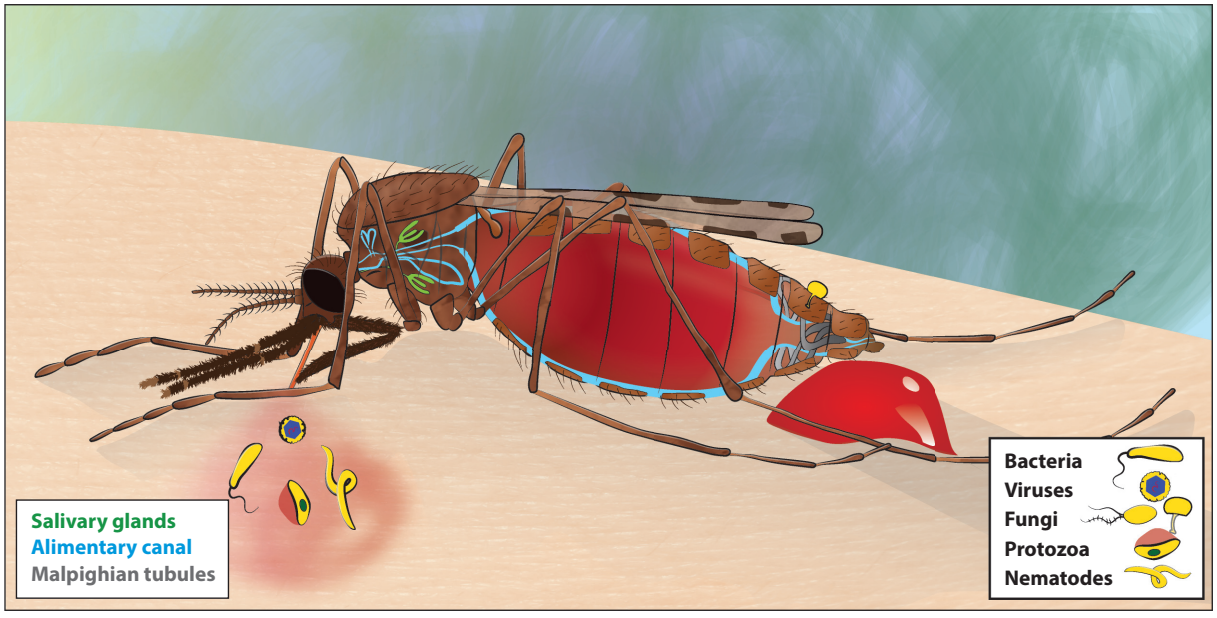

Figure 2

The routes of infection in adult mosquitoes. Adult mosquitoes are exposed to a number of entomopathogens, including viruses, bacteria, and fungi, in their environment while sugar feeding, resting, or ovipositing. In addition, female mosquitoes, represented by the *Anopheles gambiae* depicted in this image, are potentially exposed to blood-borne pathogens, including viruses, protozoa, and filarial nematodes, while feeding on their mammalian host. Current knowledge suggests that the vast majority of pathogens infect via the oral route. In addition, entomopathogenic fungi such as *Beauveria bassiana* or *Metarhizium anisopliae* may penetrate the cuticle directly after the spores are picked up by contact with the cuticle. For references and further information, please see Section 2. Image credit: Victoria S. Rhodes.

2.3.2. *Plasmodium* parasites. Many species of *Plasmodium* parasites are transmitted by mosquitoes; the best studied of these are transmitted by *Anopheles* species. In a susceptible mosquito host, gametocyte-stage parasites are ingested with a blood meal (see **Figure 2**). The midgut environs trigger exflagellation and production of male and female gametes that undergo sexual reproduction in the gut lumen. The end product is a motile ookinete that invades midgut epithelial cells and proves lethal to cells in the migration path. These cells are repaired by an actin-driven restitution mechanism (79, 161). In the extracellular space of the subepithelial basal labyrinth, the parasites undergo meiotic and mitotic divisions in the oocyst form to produce sporozoites. These break out of the oocyst (129), traverse the hemocoel with the flow of hemolymph (83), and invade the salivary glands with seemingly little to no pathological impact (135).

2.3.3. Nematodes. Filarial worms that are transmitted by mosquitoes inflict more damage on the mosquito than do the other MBPs. Here, the pathobiology of infection is described for the dog heartworm parasite, *Dirofilaria immitis*. Mosquitoes ingest microfilaria-stage parasites from the blood of an infected host (see **Figure 2**). Microfilariae traverse the midgut lumen, migrate up the Malpighian tubules, and become intracellular within the distal cells. Parasites transform into first-stage larvae, molt to the second stage (L2), and begin to actively feed. Parasites go from 250 to 950 μM in length in approximately 10 days—all within primary cells (132). When development is complete, L3s break out and migrate through the head and into and out of the proboscis for transmission. The primary cells witness significant mechanical damage as the nematodes enter, grow, and exit (132). With high parasite burdens, infection results in increased mortality of the

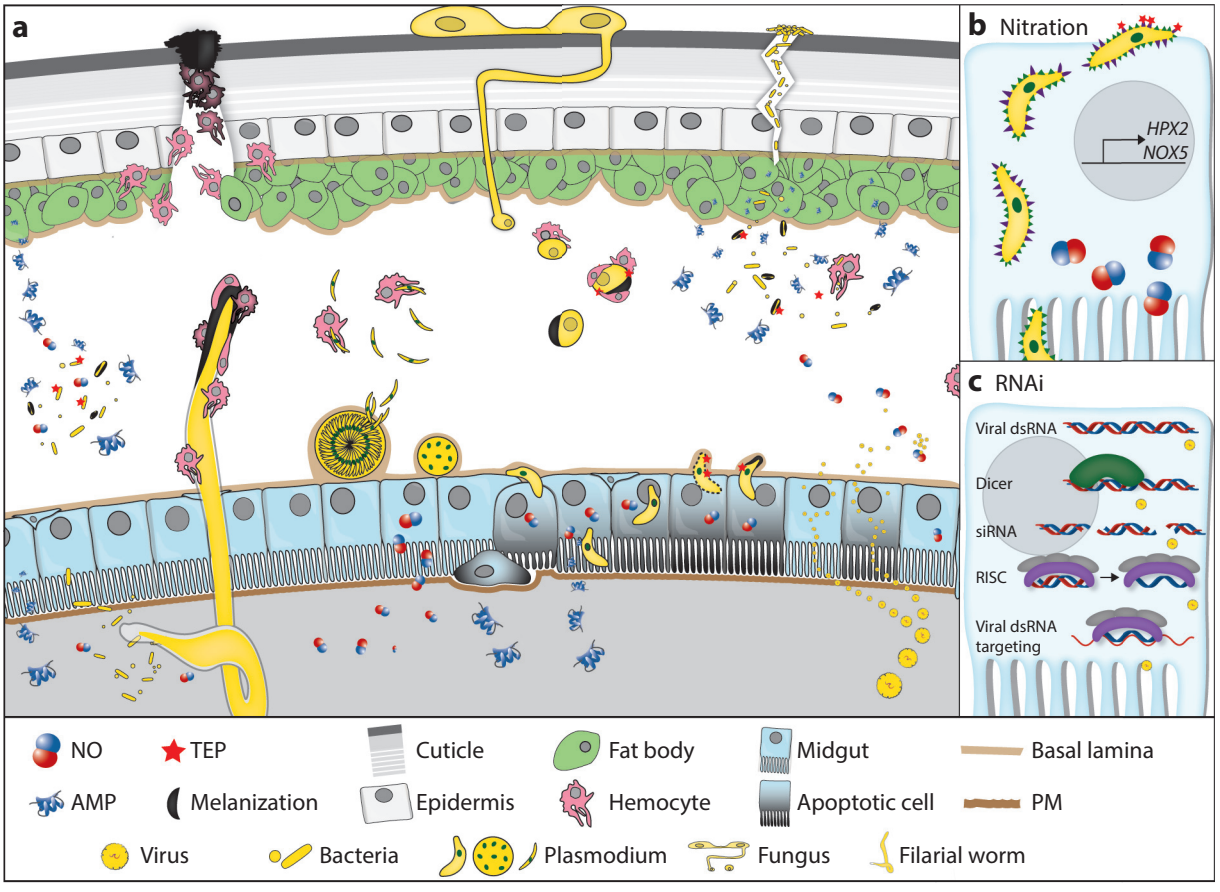

Figure 3

(*a*) Mosquito immune reactions against incoming pathogens. Mosquitoes mount humoral, cellular, and intracellular immune reactions against the range of pathogens they encounter as larvae or adults (discussed in detail in Section 3). Major immune-competent tissues in mosquitoes are epithelia such as the midgut, the fat body, and hemocytes. Antimicrobial peptides (AMPs) are produced by all three of these tissues in response to infection with bacteria, fungi, and eukaryotic parasites as well as the damage ensued by injury. AMPs secreted into the hemocoel and midgut lumen affect both bacteria and the ookinete stage of malaria parasites. Melanization is crucial in the process of wound healing, under certain conditions limits filarial worm and malaria infections, and is also mounted against bacteria. Hemocytes are the sole source of key enzymes for melanization, are critical for wound healing, and are critical for phagocytosis of both bacteria and sporozoite-stage *Plasmodium* parasites, while forming loose capsules around filarial worms as well as blastospores and hyphal bodies of fungi in the hemocoel. Midgut epithelial cells provide important intracellular immune responses that limit pathogen entry. (*b*) Malaria ookinetes may be marked for destruction by nitration of their surface through the action of two key enzymes, heme peroxidase (HPX2) and NADPH oxidase 5 (NOX5). (*c*) Virus infections are impeded by RNA interference (RNAi) through cleavage of dsRNA genomes or replication intermediates. Finally, midgut, fat body, and hemocytes are producers of nitric oxide (NO), which itself is toxic and limits bacterial and malaria infections. In addition, it may serve as a signaling molecule within the innate immune system, linking humoral, cellular, and epithelial immunity. Abbreviations: dsRNA, double-stranded RNA; PM, peritrophic matrix; RISC, RNA-induced silencing complex; siRNA, small interfering RNA; TEP, thioester-containing protein. Image credit: Victoria S. Rhodes.

mosquito. Interestingly, microfilariae rarely infect all five of the Malpighian tubules (44). From the host perspective, the parasite burden can be limited by an active melanization immune response that limits the parasite load, as is seen in *Ae. trivittatus* infected with *D. immitis* (44).

A parallel course is taken for *Wuchereria bancrofti* and *Brugia* species of parasite that cause lymphatic filariasis, wherein microfilariae-stage parasites penetrate the midgut epithelium, traverse the hemocoel, and take on an intracellular existence in the indirect flight muscles (64). Worms invade individual myofibers and grow and molt as described above. The thoracic musculature of *Armigeres subalbatus* does not appear diseased as a result of the infection process with *Brugia pahangi*, and no obvious voids are left in the musculature as worms migrate through and out of the tissue (5).

3. MECHANISTIC PERSPECTIVES ON MOSQUITO IMMUNITY

Much attention has focused on the immune repertoire deployed against MBPs, especially malaria parasites and mosquito-borne viruses, which has been reviewed elsewhere (e.g., 128, 171). Brief descriptions of humoral, cellular, and intracellular immunity effector mechanisms are provided for MBP and commensal and entomopathogenic interactions wherever possible.

3.1. Humoral Immunity

Symbionts and pathogens face an onslaught of humoral immune effectors in the midgut lumen and hemocoel. In these spaces, there is threat from antimicrobial peptides, production of eumelanin, and elements of the complement-like pathway.

3.1.1. Antimicrobial peptides. Antimicrobial peptides (AMPs) are small charged peptides that were characterized in extracts of hemolymph that had demonstrable antibacterial and antifungal activity ex vivo. The suite of AMPs that is encoded in mosquito genomes includes defensins, cecropins, attacins, holotricin, and the mosquito-specific gambicin (168). There is little doubt that these are potent, inducible infection-response genes that play an important role in overall innate immune capacity. Interestingly, although these peptides have distinct activity against bacterial types in vitro, the genes are strongly induced by a number of types of pathogens. *Ae. aegypti* defensin, for example, is inducible upon septic infection with bacteria and during infection with *B. malayi* and *D. immitis*, and it is abundant in callow pupae (11).

Coggins et al. (53) noted that *Ae. aegypti* is better equipped to survive septic bacterial infection than *An. gambiae*, and this correlates with increased transcriptional induction of AMPs and other humoral factors. This plays out at the peptide level too; in *Ae. aegypti*, defensin concentrations in the hemolymph can reach 45 μM (113). In *An. gambiae*, defensins reach a range of 1 to 5 μM (143). Additionally, suppression of *Ae. aegypti* defensin does not impact mosquito survivorship postinfection, and many bacteria in a septic infection are cleared before defensin expression reaches its peak (11). In contrast, *An. gambiae* mosquitoes challenged with high doses of *Staphylococcus aureus* succumb to infection after defensin knockdown (23).

The anterior midgut of *An. gambiae* larvae and adults constitutively expresses antimicrobial peptides (125, 166). Commensal bacteria in the mosquito midgut likely continuously encounter AMPs that shape the community structure of the midgut microbiome. Indeed, knockdown of AMPs in *An. gambiae* increases midgut bacterial counts (59).

Blood-meal-induced expression of cecropin A and defensin A in septic systemic infection of *Ae. aegypti* lowered the colony-forming units of *P. aeruginosa* and increased survivorship (103).

The role and impact of AMPs also has been explored for *B. bassiana* infections. Expression of antimicrobial peptides is strongly upregulated upon *B. bassiana* infection (60, 152).

Finally, AMPs significantly affect survival of several MBPs, and augmenting AMP production is a provocative strategy for reducing MBP transmission. Defensin injected into the hemocoel of *Ae. aegypti* kills oocysts and hemolymph sporozoites of *P. gallinaceum* (149), and septic injury dramatically reduces infection prevalence and intensity of *B. malayi* and *P. gallinaceum* in *Ae. aegypti* (111, 112). Blood-meal-induced expression of *An. gambiae* cecropin A in the midgut of transgenic mosquitoes reduces the *P. berghei* parasite load (99). Similar results were obtained using blood-meal-induced expression of cecropin A and defensin A (103).

3.1.2. Melanization. Melanization is a biochemically conserved process but is uniquely used by arthropods as an immune response mechanism to contain and kill foreign invaders (39, 47). Melanization is thought to kill pathogens through nutrient starvation and/or direct toxic effects of reaction intermediates and by-products (42, 123).

The biochemical pathway that leads to melanization involves enzymatic and nonenzymatic reactions that convert tyrosine to 5,6-dihydroxyindole (DHI) to eumelanin (reviewed in Reference 122). Monophenoloxygenase phenoloxidase (PO) is the key enzyme of this pathway (123). Although most insect genomes encode two to three POs, mosquito genomes encode nine to ten prophenoloxidase (proPO) systems with high sequence similarity (12, 124, 168), each with distinct expression patterns (2, 119), implying functional differences. Mosquito proPO systems are highly expressed in hemocytes (84, 119), thereby linking cellular and humoral immunity.

Melanization occurs readily in the hemolymph, the extracellular space of the subepithelial basal membrane labyrinth of the midgut, and the integument but not the midgut lumen. It is plausible that commensal midgut bacteria avoid this immune reaction through compartmentalization and sequestration. Small melanotic capsules are sometimes observed in the midgut subepithelial labyrinth of *An. gambiae* mosquitoes, which may result from intestinal bacteria breaching the midgut epithelium during blood feeding.

In contrast to the microbial gut flora of mosquitoes, melanization of entomopathogens is readily observed. The penetration path through the larval cuticle by the ciliate *T. clarki* (see Section 2.2.1) is visible as black spots, revealing wound healing by melanization. Ultrastructural examination of the infection revealed some extensive melanization in resistant mosquitoes and no melanization in highly infected individuals (49). Similarly, infections by entomopathogenic fungi elicit a melanization response. Hyphae of *L. giganteum* in *Ae. aegypti* larvae or *B. bassiana* in adult *An. gambiae* are partially melanized in the hemocoel and/or the integument (30, 174). The robustness of the melanization response correlates positively with better survivorship in larval infections of *Ae. aegypti*, *Cx. pipiens*, and *An. gambiae*. Although 99% of *Aedes* and *Culex* larvae succumb to the infection, 44% of *An. gambiae* larvae survive (74). Thus, melanization may be a resistance mechanism in this interaction and places strong pressure on the entomopathogen to evade or suppress this immune response.

Melanization also has received considerable attention, as it is a phenotype often observed of nonpermissive infections in several MBP–mosquito vector species combinations (18, 55, 66, 78). In addition, it is a readily selectable phenotype of refractoriness to allochthonous malaria and filarial worm parasites in otherwise permissive species combinations (43, 54, 93). Like entomopathogens, MBP must either actively suppress melanization or employ evasion strategies, both of which have been observed (28, 114).

3.1.3. The complement-like pathway. In *An. gambiae*, the complement-like pathway results in activation and deposition of a thioester-containing protein 1 (TEP1), a complement-like opsonin

that is structurally related to vertebrate C3 complement factor (14, 106). The pathway consists of at least four additional players, including two leucine-rich repeat proteins, APL1C (144) and LRIM1 (130), and two clip domain serine protease homologs, SPCLIP1 (139) and CLIPA2 (173). TEP1 contains a highly reactive thioester-binding motif that enables covalent binding to a wide variety of substrates. The current model of the complement-like pathway goes as follows: Upon initial proteolytic cleavage of TEP1 in the hemolymph, TEP1 is stabilized as a heterotrimer bound to APL1C and LRIM1 (68, 140). Deposition of cleaved TEP1 on the microbial surface triggers the formation of a TEP1 convertase, which rapidly opsonizes by recruiting full-length TEP1 molecules from the hemolymph and converts it to the active form on the microbial surface. TEP1 accumulation is regulated positively by SPCLIP1 (139) and negatively by CLIPA2 (173). Activation of the complement pathway may ultimately lead to killing by lysis. Ookinetes subjected to opsonization show evidence of lysis, including membrane blebbing, fragmentation, and the release of hemozoin granules (24, 160). The molecular specifics of lysis are currently not understood, so it is unclear whether TEP1 merely initiates lysis and/or executes lysis by killing the parasite.

The extent to which the complement-like pathway affects the midgut microbial flora in mosquito larvae and adults is unknown. Transcriptomic studies show that members of the complement-like pathway, including TEPs, are expressed in the midgut of adult mosquitoes (91, 125, 164), suggesting that commensal microorganisms may encounter the complement-like pathway. In addition, knockdown of TEP1 significantly increases the number of CFUs in *An. gambiae* (59).

Data on the role of the complement-like pathway in infections with entomopathogenic infections are starting to emerge. TEP1 binds to hyphae of *B. bassiana* growing in the hemolymph of adult *An. gambiae*. Knockdown of TEP1 in *An. gambiae* mosquitoes almost abolishes melanization of growing hyphae, increases hyphal growth, and decreases mosquito survival (174). Similarly, knockdown of TEP22 in *Ae. aegypti* decreases survival of adult mosquitoes infected with *B. bassiana* (164). These data confirm that, at least for *B. bassiana*, infection engages the complement-like pathway and, in turn, melanization. However, rapid hyphal growth seems to outrun melanization, as proPO is readily detectable on the apical parts of the hyphae, but no melanization is observed (174). This observation may explain not only why *B. bassiana*–infected mosquitoes ultimately succumb to infection but also why distinct fungal isolates vary in their ability to rapidly kill mosquito hosts (27, 158).

The effects of the complement-like pathway on MBPs has been explored extensively in the context of malaria parasites, where it constitutes a major ookinete-killing mechanism dependent on the genetic background of the mosquito and parasite (22). In terms of the genetics of the parasite, variation in the susceptibility to *Plasmodium falciparum* depends in part on the Psf47 gamete surface protein (117) but does not fully explain the range of immune evasions observed in experimental infections (62). In addition, infection outcome is influenced by allelic variants of TEP1 (25). Whether the complement-like pathway is required for melanization of filarial worms (see section above) is unknown. Given that opsonization by TEP1 is key to melanization of bacteria, fungi, and *Plasmodium* parasites, the likelihood of a comparable role in filarial worm infection is high.

3.2. Cellular Immunity

Cellular immune responses in mosquitoes are executed by hemocytes, the primary immune cells that circulate in the hemolymph. In contrast to humoral immune reactions, cellular immunity provides immediate responses that are triggered and executed within minutes of pathogen

exposure by virtue of phagocytosis, encapsulation, and nodulation (see **Figure 3**; reviewed in References 21 and 87). Approximately 2,000 to 5,000 hemocytes are present in an adult mosquito, but only a small proportion circulate freely (87, 101). However, infection can alter the number of hemocytes dramatically by cell proliferation. In *Ae. aegypti*, the number of hemocytes in circulation rapidly increases upon infection with *D. immitis* or *Escherichia coli* (46, 101). Although *P. berghei* infection does not increase hemocyte numbers in *An. gambiae*, (13), blood feeding itself strongly increases hemocyte proliferation, de facto providing a hemocyte-enriched hemocoel for malaria parasites crossing the midgut epithelium (31, 32, 35).

Several distinct mosquito hemocyte classifications have been proposed, but the current consensus distinguishes three cell types—granulocytes, oenocytoids, and prohemocytes (37, 87). Of the circulating hemocytes in adult mosquitoes, 80% to 95% are granulocytes. These phagocytically competent cells express many immune factors, including AMPs, members of the complement-like pathway, and PO. Oenocytoids are nonphagocytic cells and express PO at high levels. Prohemocytes were originally thought to be hemocyte progenitor cells. However, these cells were recently found to be phagocytically active, potentially arising from asymmetric cell division of differentiated granulocytes (101).

In the context of bacterial infections, it is unlikely that hemocytes interact with the gut microbiome. However, they may interact with intestinal bacteria after a blood meal if bacteria leak into the hemocoel with stretching of the midgut epithelium. Activation and proliferation of hemocytes within the first day post–blood feeding (31, 35) could be attributed to temporary bacteremia; this hypothesis awaits experimental support. Mosquito hemocytes readily phagocytize bacteria, yeast cells, and malaria sporozoites that enter the hemocoel (**Figure 3**; reviewed in Reference 87). Hemocytes also form cell aggregates around bacteria and are referred to as nodules (100, 116).

In addition, mosquito hemocytes are associated with melanotic capsules around filarial worms (44, 45), entomopathogenic fungi (174), and artificial surfaces like glass beads (114). However, capsules formed by mosquito hemocytes tend to be looser and less stratified than capsules observed in other insects, and it is likely that encapsulated parasites die as a result of melanization of the capsule. In addition, hemocytes contribute to wound healing by release of proPO and rapid phagocytosis of invasive bacteria (**Figure 3**) (105).

Hemocytes likely also play a role in arbovirus immunobiology in the mosquito host. Circulating hemocytes in several *Aedes* and *Culex* species rapidly take up and serve as a site of replication for Sindbis virus (134). Therefore, hemocytes may serve a role in shuttling viruses through the hemocoel or in achieving a replication hurdle to infect the salivary glands, as was predicted previously (80). Hemocytes also could play a role in the salivary gland infection barrier. Hemolymph titers of Western equine encephalitis virus titers are significantly higher in susceptible, as compared to refractory, *Cx. tarsalis* (80).

Interestingly, although hemocytes do not physically interact with malaria parasites in the midgut, these cells express and secrete several key agonists and antagonists that hinder ookinete and oocyst development, including opsonins, PO, antimicrobial peptides, and nitric oxide (13, 86, 109, 110, 136). Delivery of these molecules to the site of infection may involve localized release of microvesicles from hemocytes (36). These cells contribute greatly to the developmental bottleneck encountered during midgut passage (75, 155).

Although the different branches of the humoral, cellular, and intracellular immune systems are presented here as distinct entities, they often act in concert on the same organism across the range of microbial interactions. For example, defensin colocalizes with PO on melanized *Micrococcus luteus* bacteria in the hemocoel of *Ae. aegypti* (85). TEP1 colocalizes with PO on the surface of melanized rodent malaria parasites in the midgut of *An. gambiae* (24) and most likely also does so on the surface of *B. bassiana* hyphae and blastospores, as TEP1 is required for their melanization

and most likely hemocyte encapsulation (174). This integration occurs at least partially during processes upstream of the immune effector mechanisms, including the activation of immune signal transduction pathways and proteolytic cascades.

3.3. Intracellular Immunity

In addition to humoral and cellular effector systems, the mosquito immune system deploys intracellular effector mechanisms, including programmed cell death (PCD) and RNA interference (RNAi). The following section highlights specific examples of intracellular immune mechanisms in the context of infection with intracellular entomopathogens and MBPs that are transiently or permanently intracellular during infection.

3.3.1. Programmed cell death. Two types of PCD have been described during infection in mosquito hosts: apoptosis and autophagy. Apoptosis leads to cell shrinking and blebbing and is evidenced by chromatin condensation and DNA fragmentation, as well as caspase activity. Autophagy is characterized by the formation of large autophagic vacuoles; unlike apoptosis, autophagy is not characterized by caspase activity or chromatin condensation and does not always equate to cell death.

Apoptosis as a function of innate immunity to MBPs has been explored as a response to malaria parasites in *Anopheles* mosquitoes (92). Midgut cells of *An. stephensi* or *An. gambiae* infected with *P. berghei* ookinetes undergo apoptosis and are expelled into the midgut lumen. Parasite infection is terminated unless the ookinete migrates quickly through the epithelium and escapes apoptotic cells (79, 162). Even if escape is timely, ookinetes experience oxidative stress caused by apoptosis, which causes nitration of proteins within midgut epithelial cells (104). Nitration of parasite surface proteins could mark the parasite for destruction through the complement system once it reaches the basal labyrinth of the midgut epithelium (**Figure 3**; 127). Functional genomics analysis of *An. stephensi* infected with *P. berghei* malaria parasites shows that caspase E497 is upregulated during midgut infection, and E517 caspase and a bax-like inhibitor of apoptosis peak in expression as the parasites burst out of the gut (172). Although the ovaries are not infected during malaria parasite development, *P. yoelii* infection causes a significant decrease in the number of developing follicles, and apoptosis in the atretic follicles is evident (3, 89). Autophagy may also play a role in dictating the outcome of *An. stephensi–P. falciparum* infection. Overexpression of peptide phosphatase and tensin homolog, an inhibitor of the insulin/insulin-like growth factor signaling cascade, increases expression of autophagy genes and significantly decreases *P. falciparum* oocyst prevalence and intensity (81).

The extent to which apoptosis functions in immunity to arbovirus infection in the mosquito host, as opposed to a strategy manipulated by the virus for dissemination, is not clear (52, 69). Arbovirus infection certainly can induce apoptosis in vivo in mosquito hosts. *Cx. quinquefasciatus* infected with West Nile virus (WNV) show ultrastructural evidence of apoptosis in the salivary glands (71, 72). Apoptotic activity also is evident in salivary glands of *Ae. albopictus* infected with Sindbis virus (SINV) (97, 157) and *Ae. aegypti* infected with Chikungunya virus (58). In the context of apoptosis as an infection-limiting response, caspase expression was higher in the midgut of a DENV refractory strain of *Ae. aegypti* (126), and hallmarks of apoptotic activity are evident in midgut epithelial cells of a WNV refractory strain of *Cx. pipiens* (157). RNAi suppression of the initiator caspase AeDronc results in less dissemination of SINV (163) and DENV (63); in the latter case, Eng and colleagues speculate that this effect is due to dysregulation of autophagy. Autophagy is relatively unexplored as an antiviral immune response in mosquitoes, but there are intriguing leads in *D. melanogaster* to suggest that autophagy suppresses virus replication (151).

3.3.2. RNA interference. Arguably, the first evidence of the antiviral nature of RNAi came from in vitro studies of arbovirus–mosquito interactions. Cells infected with an expression vector for an antisense segment of LaCrosse virus (LACV) were superinfected with LACV and yielded far less LACV than controls (141). This capacity was effective in suppressing infection of several species of virus, and the orientation (sense or antisense) of the sequence did not affect virus suppression (1). Hoa et al. (88) identified Dicer 2, Argonaute 2 (Ago-2), and Argonaute 3 as drivers of the RNAi response in an *Anopheles* cell line. Ago-2 suppression in *An. gambiae* increased replication and dissemination of O'nyong-nyong virus (Togaviridae) (96) and so provided conclusive evidence of RNAi as an antiviral that protects the mosquito host from uncontrolled virus replication (see **Figure 3**).

This type of Dicer-driven RNAi results in virus genome cleavage into small [21 nucleotides (nt)] interfering RNAs (siRNAs) (121). The PIWI-interacting RNAs (24–27 nt) also are produced during infection with many of the arboviruses and play an unresolved role in antiviral immunity, as reviewed recently (115). Most recently, a mechanism for tolerance and persistence has been proposed whereby arbovirus infection and innate reverse transcriptase activity produce genome-integrated viral DNAs through which the RNAi response is reinforced; suppression of reverse transcriptase activity in *Ae. aegypti* infected with either CHIKV or DENV results in increased susceptibility to infection (73).

4. IMMUNITY IN THE CONTEXT OF THE MOSQUITO HOLOBIONT

4.1. Effect of the Resident Microbiota on Mosquito-Borne Pathogen Transmission

The resident microbiota has significant impact on the outcome of infections across all kinds of multicellular organisms. In mosquitoes, intestinal bacteria influence the development of several MBPs, including viruses and malaria parasites (51, 57, 82, 95). In *An. gambiae*, dysbiosis induced by feeding an antibiotic cocktail significantly increases the number of developing *P. falciparum* oocysts. This effect was abolished in mosquitoes with a reconstituted microbiota, demonstrating that the effect on infection was not due to off-target effects of antibiotic treatment (59). Similarly, experimental dysbiosis in *Ae. aegypti* leads to higher DENV (142) and SINV titers (8). In contrast, ONNV infection in *An. gambiae* partially depends on an intact microbiota, as experimental dysbiosis reduces virus titers (34). Multiple mechanisms mediate the effect of dysbiosis on viral infection, including resource competition and vector immunity (reviewed in References 57 and 82). Dysbiosis significantly reduces expression of immunity genes in the midgut, including AMPs.

In turn, MBP infection can have a reciprocal impact on the resident microbiota, as seen in *Ae. triseriatus* and *Ae. japonicus* (120), where infection with LACV decreased operational taxonomic unit (OTU) richness and evenness of resident fungi, whereas bacterial OTUs increased. In *Ae. albopictus*, CHIKV infection significantly altered relative intensities of Gammaproteobacteria, especially within the Enterobacteriaceae (176). Interestingly, DENV causes the only known MBP infection that significantly reduces the midgut bacterial load as determined by 16S sequencing (142). Whether any of these microbiome shifts are due to the impact of viral infection on the immune system is currently unknown.

4.2. The Impact of Coinfection on Mosquito-Borne Pathogen Transmission

The deleterious effects of the microbiome on MBP infections levels has been interpreted as immune priming, where the microbiota continuously provides a priming event, which elicits a

low-level immune response that increases the efficacy of anti-MBP immunity (57). The effects of immune priming by experimentally induced septic bacterial infections have been explored in a number of MBP–mosquito species combinations. Systemic *E. coli* and *M. luteus* infection in *An. gambiae* and *Ae. aegypti* significantly induced a humoral immune response and reduced oocyst levels of *P. berghei* and *P. gallinaceum*, respectively (112). The same experimental setup reduced infection of *Ae. aegypti* with *B. malayi* filarial worms (111). However, experimental infections of immune-primed *Cx. pipiens* with field-collected *W. bancrofti* did not augment infection intensity or prevalence (10). In addition to septic bacterial infections, priming using the same MBP can reduce the infectious burden of subsequent infections, as observed for *P. berghei* and *P. falciparum* infections in *An. gambiae* (145); this effect is dependent on the presence of an intact microbiota.

The most dramatic impact on MBP transmission has been achieved by transfections with the rickettsial endosymbiont *Wolbachia pipientis* (reviewed in Reference 33). Transinfection with the *w*ALb strain of *Wolbachia* blocks *P. berghei* and *P. falciparum* infections in *An. stephensi* (20, 94), and the *w*MelPop strain blocks *P. gallinaceum* infections in *Ae. aegypti* (118). *An. gambiae* and *An. coluzzii* thus far have been impervious to transinfections. Naturally occurring infections with the *w*Anga strain of *Wolbachia* are negatively correlated with *P. falciparum* infection in field-collected *An. coluzzii* (150). Although the *w*Anga strain lacks the ability to induce cytoplasmatic incompatibility, which limits its utility as a control agent for malaria, its discovery emphasizes the potential for the malaria parasite–blocking ability of *Wolbachia* (150). Transinfections of several *Wolbachia* strains into *Ae. aegypti* and *Ae. albopictus* impair viral MBP infection and transmission, including DENV, CHIK, YFV, and WNV, and are influenced by the combination *Wolbachia* strain, mosquito vector, and virus (Reference 33 and references therein). Transinfection with the *w*Mel strain also protected *Ae. aegypti* from ZIKV (6, 61). *Ae. aegypti* mosquitoes transinfected with the *w*Mel strain are currently being used in multiple Southeast Asian and South American countries to reduce DENV, CHIKV, and ZIKV disease burden (reviewed in Reference 33). Initially, immune priming was proposed as the underlying mechanism of *Wolbachia* pathogen interference. However, recent findings have brought this notion into question, and data from *Drosophila* suggest viral replication as a target of pathogen interference (19). Whether these findings transfer to mosquitoes is currently unclear, and the search for the molecular underpinnings of this phenomenon continues.

5. CONCLUSIONS

The mosquito holobiont encompasses organisms that passively populate, aggressively infect and cause disease, or infect and use mosquitoes as a vehicle for transmission. The sum of these interactions has shaped the mosquito immune system to maximize mosquito survival and minimize deleterious side effects of overly zealous immune responses. As a result, the mosquito immune system consists of effector mechanisms that defy narrow specificity in favor of cleverly co-opting physiology. Not only MBPs but also commensals and entomopathogens must strike an arrangement that allows them to survive and thrive in the context of mosquito immunity. The field is ripe with opportunity to understand immune response capacity in mosquitoes beyond the canonical immune responses that are well described only for MBP–vector interactions.

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LITERATURE CITED

1. Adelman ZN, Blair CD, Carlson JO, Beaty BJ, Olson KE. 2001. Sindbis virus-induced silencing of dengue viruses in mosquitoes. *Insect Mol. Biol.* 10(3):265–73
2. Ahmed A, Martin D, Manetti AG, Han S-J, Lee W-J, et al. 1999. Genomic structure and ecdysone regulation of the prophenoloxidase 1 gene in the malaria vector *Anopheles gambiae*. *PNAS* 96(26):14795–800
3. Ahmed AM, Hurd H. 2006. Immune stimulation and malaria infection impose reproductive costs in *Anopheles gambiae* via follicular apoptosis. *Microbes Infect.* 8(2):308–15
4. Akorli J, Gendrin M, Pels NAP, Yeboah-Manu D, Christophides GK, Wilson MD. 2016. Seasonality and locality affect the diversity of *Anopheles gambiae* and *Anopheles coluzzii* midgut microbiota from Ghana. *PLOS ONE* 11(6):e0157529
5. Aliota MT, Fuchs JF, Rocheleau TA, Clark AK, Hillyer JF, et al. 2010. Mosquito transcriptome profiles and filarial worm susceptibility in *Armigeres subalbatus*. *PLOS Negl. Trop. Dis.* 4(4):e666
6. Aliota MT, Peinado SA, Velez ID, Osorio JE. 2016. The *w*Mel strain of *Wolbachia* reduces transmission of Zika virus by *Aedes aegypti*. *Sci. Rep.* 6:28792
7. Andreadis TG. 2005. Evolutionary strategies and adaptations for survival between mosquito-parasitic microsporidia and their intermediate copepod hosts: a comparative examination of *Amblyospora connecticut* and *Hyalinocysta chapmani* (Microsporidia: Amblyosporidae). *Folia Parasitol.* 52(1–2):23–35
8. Barletta ABF, Nascimento-Silva MCL, Talyuli OAC, Oliveira JHM, Pereira LOR, et al. 2017. Microbiota activates IMD pathway and limits Sindbis infection in *Aedes aegypti*. *Parasites Vectors* 10(1):103
9. Barros FSM, Vasconcelos SD, Arruda ME, Confalonieri UEC, Luitgards-Moura JF, Honório NA. 2006. Tetrahymenidae infection in mosquito populations in a malaria-endemic region of the Amazon. *J. Invertebr. Pathol.* 91:199–201
10. Bartholomay LC, Farid HA, Ramzy RM, Christensen BM. 2003. *Culex pipiens*: characterization of immune peptides and the influence of immune activation on development of *Wuchereria bancrofti*. *Mol. Biochem. Parasitol.* 130(1):43–50
11. Bartholomay LC, Fuchs JF, Cheng L, Beck ET, Vizioli J, et al. 2004. Reassessing the role of defensin in the innate immune response of the mosquito, *Aedes aegypti*. *Insect Mol. Biol.* 13(2):125–32
12. Bartholomay LC, Waterhouse RM, Mayhew GF, Campbell CL, Michel K, et al. 2010. Pathogenomics of *Culex quinquefasciatus* and meta-analysis of infection responses to diverse pathogens. *Science* 330(6000):88–90
13. Baton LA, Robertson A, Warr E, Strand MR, Dimopoulos G. 2009. Genome-wide transcriptomic profiling of *Anopheles gambiae* hemocytes reveals pathogen-specific signatures upon bacterial challenge and *Plasmodium berghei* infection. *BMC Genom.* 10(1):257
14. Baxter RH, Chang C-I, Chelliah Y, Blandin S, Levashina EA, Deisenhofer J. 2007. Structural basis for conserved complement factor-like function in the antimalarial protein TEP1. *PNAS* 104(28):11615–20
15. Becnel JJ, White SE, Moser BA, Fukuda T, Rotstein MJ, et al. 2001. Epizootiology and transmission of a newly discovered baculovirus from the mosquitoes *Culex nigripalpus* and *C. quinquefasciatus*. *J. Gen. Virol.* 82(2):275–82

16. Becnel JJ, White SE, Shapiro AM. 2003. *Culex nigripalpus* nucleopolyhedrovirus (CuniNPV) infections in adult mosquitoes and possible mechanisms for dispersal. *J. Invertebr. Pathol.* 83(2):181–83
17. Becnel JJ, White SE, Shapiro AM. 2005. Review of microsporidia-mosquito relationships: from the simple to the complex. *Folia Parasitol.* 52(1–2):41–50
18. Beerntsen BT, Luckhart S, Christensen BM. 1989. *Brugia malayi* and *Brugia pahangi*: inherent difference in immune activation in the mosquitoes *Armigeres subalbatus* and *Aedes aegypti*. *J. Parasitol.* 75(1):76–81
19. Bhattacharya T, Newton ILG, Hardy RW. 2017. *Wolbachia* elevates host methyltransferase expression to block an RNA virus early during infection. *PLOS Pathog.* 13(6):e1006427
20. Bian G, Joshi D, Dong Y, Lu P, Zhou G, et al. 2013. *Wolbachia* invades *Anopheles stephensi* populations and induces refractoriness to *Plasmodium* infection. *Science* 340(6133):748–51
21. Blandin SA, Levashina EA. 2007. Phagocytosis in mosquito immune responses. *Immunol. Rev.* 219:8–16
22. Blandin SA, Marois E, Levashina EA. 2008. Antimalarial responses in *Anopheles gambiae*: from a complement-like protein to a complement-like pathway. *Cell Host Microbe* 3(6):364–74
23. Blandin SA, Moita LF, Köcher T, Wilm M, Kafatos FC, Levashina EA. 2002. Reverse genetics in the mosquito *Anopheles gambiae*: targeted disruption of the *Defensin* gene. *EMBO Rep.* 3(9):852–56
24. Blandin SA, Shiao S-H, Moita LF, Janse CJ, Waters AP, et al. 2004. Complement-like protein TEP1 is a determinant of vectorial capacity in the malaria vector *Anopheles gambiae*. *Cell* 116(5):661–70
25. Blandin SA, Wang-Sattler R, Lamacchia M, Gagneur J, Lycett G, et al. 2009. Dissecting the genetic basis of resistance to malaria parasites in *Anopheles gambiae*. *Science* 326(5949):147–50
26. Blanford S, Chan BHK, Jenkins N, Sim D, Turner RJ, et al. 2005. Fungal pathogen reduces potential for malaria transmission. *Science* 308(5728):1638–41
27. Blanford S, Jenkins NE, Read AF, Thomas MB. 2012. Evaluating the lethal and pre-lethal effects of a range of fungi against adult *Anopheles stephensi* mosquitoes. *Malar. J.* 11(1):365
28. Boete C, Paul RE, Koella JC. 2004. Direct and indirect immunosuppression by a malaria parasite in its mosquito vector. *Proc. Biol. Sci.* 271(1548):1611–15
29. Bolling BG, Weaver SC, Tesh RB, Vasilakis N. 2015. Insect-specific virus discovery: significance for the arbovirus community. *Viruses* 7(9):4911–28
30. Brey PT, Lebrun RA, Papierok B, Ohayon H, Vennavalli S, Hafez J. 1988. Defense reactions by larvae of *Aedes aegypti* during infection by the aquatic fungus *Lagenidium giganteum* (Oomycete). *Cell Tissue Res.* 253(1):245–50
31. Bryant WB, Michel K. 2014. Blood feeding induces hemocyte proliferation and activation in the African malaria mosquito, *Anopheles gambiae* Giles. *J. Exp. Biol.* 217(8):1238–45
32. Bryant WB, Michel K. 2015. *Anopheles gambiae* hemocytes exhibit transient states of activation. *Dev. Comp. Immunol.* 55:119–29
33. Caragata EP, Dutra HLC, Moreira LA. 2016. Exploiting intimate relationships: controlling mosquito-transmitted disease with *Wolbachia* 32(3):207–18
34. Carissimo G, Pondeville E, McFarlane M, Dietrich I, Mitri C, et al. 2015. Antiviral immunity of *Anopheles gambiae* is highly compartmentalized, with distinct roles for RNA interference and gut microbiota. *PNAS* 112(2):E176–85
35. Castillo JC, Brown MR, Strand MR. 2011. Blood feeding and insulin-like peptide 3 stimulate proliferation of hemocytes in the mosquito *Aedes aegypti*. *PLOS Pathog.* 7(10):e1002274
36. Castillo JC, Ferreira ABB, Trisnadi N, Barillas-Mury C. 2017. Activation of mosquito complement antiplasmodial response requires cellular immunity. *Sci. Immunol.* 2(7):1–10
37. Castillo JC, Robertson AE, Strand MR. 2006. Characterization of hemocytes from the mosquitoes *Anopheles gambiae* and *Aedes aegypti*. *Insect Biochem. Mol. Biol.* 36(12):891–903
38. Cator LJ, George J, Blanford S, Murdock CC, Baker TC, et al. 2013. "Manipulation" without the parasite: Altered feeding behaviour of mosquitoes is not dependent on infection with malaria parasites. *Proc. Biol. Sci.* 280(1763):20130711
39. Cerenius L, Lee BL, Söderhäll K. 2008. The proPO-system: pros and cons for its role in invertebrate immunity. *Trends Immunol.* 29(6):263
40. Chapman HC. 1974. Biological control of mosquito larvae. *Annu. Rev. Entomol.* 19:33–59
41. Charles JF, de Barjac H. 1983. Action of crystals of *Bacillus thuringiensis* var. *israelensis* on the midgut of *Aedes aegypti* L. larvae, studied by electron microscopy. *Ann. Microbiol.* 134A(2):197–218

42. Chen CC, Chen CS. 1995. *Brugia pahangi*: effects of melanization on the uptake of nutrients by microfilariae in vitro. *Exp. Parasitol.* 81(1):72–78
43. Chen CC, Laurence BR. 1987. Selection of a strain of *Anopheles quadrimaculatus* with high filaria encapsulation rate. *J. Parasitol.* 73(2):418–19
44. Christensen BM. 1981. Observations on the immune response of *Aedes trivittatus* against *Dirofilaria immitis*. *Trans. R. Soc. Trop. Med. Hyg.* 75(3):439–43
45. Christensen BM, Forton KF. 1986. Hemocyte-mediated melanization of microfilariae in *Aedes aegypti*. *J. Parasitol.* 72(2):220–25
46. Christensen BM, Huff BM, Miranpuri GS, Harris KL, Christensen LA. 1989. Hemocyte population changes during the immune response of *Aedes aegypti* to inoculated microfilariae of *Dirofilaria immitis*. *J. Parasitol.* 75(1):119–23
47. Christensen BM, Li J, Chen CC, Nappi AJ. 2005. Melanization immune responses in mosquito vectors. *Trends Parasitol.* 21(4):192–99
48. Christophides GK, Zdobnov E, Barillas-Mury C, Birney E, Blandin S, et al. 2002. Immunity-related genes and gene families in *Anopheles gambiae*. *Science* 298(5591):159–65
49. Clark TB, Brandl DG. 1976. Observations on the infection of *Aedes sierrensis* by a tetrahymenine ciliate. *J. Invertebr. Pathol.* 28(3):341–49
50. Clark TB, Kellen WR, Fukuda T, Lindegren JE. 1968. Field and laboratory studies on the pathogenicity of the fungus *Beauveria bassiana* to three genera of mosquitoes. *J. Invertebr. Pathol.* 11(1):1–7
51. Clayton AM, Dong Y, Dimopoulos G. 2014. The *Anopheles* innate immune system in the defense against malaria infection. *J. Innate Immun.* 6(2):169–81
52. Clem RJ. 2016. Arboviruses and apoptosis: the role of cell death in determining vector competence. *J. Gen. Virol.* 97(5):1033–36
53. Coggins SA, Estévez-Lao TY, Hillyer JF. 2012. Increased survivorship following bacterial infection by the mosquito *Aedes aegypti* as compared to *Anopheles gambiae* correlates with increased transcriptional induction of antimicrobial peptides. *Dev. Comp. Immunol.* 37(3–4):390–401
54. Collins FH, Sakai RK, Vernick KD, Paskewitz S, Seeley DC, et al. 1986. Genetic selection of a *Plasmodium*-refractory strain of the malaria vector *Anopheles gambiae*. *Science* 234(4776):607–10
55. Daniels CW. 1898. On transmission of proteosoma to birds by the mosquito: a report to the malaria committee of the royal society. *Proc. R. Soc. London* 64(402–11):443–54
56. Demaio J, Pumpuni CB, Kent M, Beier JC. 1996. The midgut bacterial flora of wild *Aedes triseriatus*, *Culex pipiens*, and *Psorophora columbiae* mosquitoes. *Am. J. Trop. Med. Hyg.* 54(2):219–23
57. Dennison NJ, Jupatanakul N, Dimopoulos G. 2014. The mosquito microbiota influences vector competence for human pathogens. *Curr. Opin. Insect Sci.* 3:6–13
58. Dong S, Kantor AM, Lin J, Passarelli AL, Clem RJ, Franz AWE. 2016. Infection pattern and transmission potential of chikungunya virus in two New World laboratory-adapted *Aedes aegypti* strains. *Sci. Rep.* 6:24729
59. Dong Y, Manfredini F, Dimopoulos G, Tchuinkam T, Faas B. 2009. Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLOS Pathog.* 5(5):e1000423
60. Dong Y, Morton JC Jr., Ramirez JL, Souza-Neto JA, Dimopoulos G. 2012. The entomopathogenic fungus *Beauveria bassiana* activate toll and JAK-STAT pathway-controlled effector genes and anti-dengue activity in *Aedes aegypti*. *Insect Biochem. Mol. Biol.* 42(2):126–32
61. Dutra HLC, Rocha MN, Dias FBS, Mansur SB, Caragata EP, Moreira LA. 2016. *Wolbachia* blocks currently circulating Zika virus isolates in Brazilian *Aedes aegypti* mosquitoes 19(6):771–74
62. Eldering M, Morlais I, van Gemert G-J, van de Vegte-Bolmer M, Graumans W, et al. 2016. Variation in susceptibility of African *Plasmodium falciparum* malaria parasites to TEP1 mediated killing in *Anopheles gambiae* mosquitoes. *Sci. Rep.* 6:20440
63. Eng MW, van Zuylen MN, Severson DW. 2016. Apoptosis-related genes control autophagy and influence DENV-2 infection in the mosquito vector, *Aedes aegypti*. *Insect Biochem. Mol. Biol.* 76:70–83
64. Erickson SM, Thomsen EK, Keven JB, Vincent N, Koimbu G, et al. 2013. Mosquito-parasite interactions can shape filariasis transmission dynamics and impact elimination programs. *PLOS Negl. Trop. Dis.* 7(9):e2433

65. Federici BA. 1995. The future of microbial insecticides as vector control agents. *J. Am. Mosq. Control Assoc.* 11(2):260–68
66. Flatt T, Heyland A, Rus F, Porpiglia E, Sherlock C, et al. 2008. Hormonal regulation of the humoral innate immune response in *Drosophila melanogaster*. *J. Exp. Biol.* 211(16):2712–24
67. Foy BD, Myles KM, Pierro DJ, Sanchez-Vargas I, Uhlířová M, et al. 2004. Development of a new Sindbis virus transducing system and its characterization in three Culicine mosquitoes and two Lepidopteran species. *Insect Mol. Biol.* 13(1):89–100
68. Fraiture M, Baxter RHG, Steinert S, Chelliah Y, Frolet CC, et al. 2009. Two mosquito LRR proteins function as complement control factors in the TEP1-mediated killing of *Plasmodium*. *Cell Host Microbe* 5(3):273–84
69. Franz AWE, Kantor AM, Passarelli AL, Clem RJ. 2015. Tissue barriers to arbovirus infection in mosquitoes. *Viruses* 7(7):3741–67
70. Garver LS, Dong Y, Dimopoulos G. 2009. Caspar controls resistance to *Plasmodium falciparum* in diverse *Anopheline* species. *PLOS Pathog.* 5(3):e1000335
71. Girard YA, Popov V, Wen J, Han V, Higgs S. 2005. Ultrastructural study of West Nile virus pathogenesis in *Culex pipiens quinquefasciatus* (Diptera: Culicidae). *J. Med. Entomol.* 42(3):429–44
72. Girard YA, Schneider BS, McGee CE, Wen J, Han VC, et al. 2007. Salivary gland morphology and virus transmission during long-term cytopathologic West Nile virus infection in *Culex* mosquitoes. *Am. J. Trop. Med. Hyg.* 76(1):118–28
73. Goic B, Stapleford KA, Frangeul L, Doucet AJ, Gausson V, et al. 2016. Virus-derived DNA drives mosquito vector tolerance to arboviral infection. *Nat. Commun.* 7:12410
74. Golkar L, Lebrun RA, Ohayon H, Gounon P, Papierok B, Brey PT. 1993. Variation of larval susceptibility to *Lagenidium giganteum* in three mosquito species. *J. Invertebr. Pathol.* 62(1):1–8
75. Gouagna LC, Bonnet S, Gounoue R, Verhave JP, Eling W, et al. 2004. Stage-specific effects of host plasma factors on the early sporogony of autologous *Plasmodium falciparum* isolates within *Anopheles gambiae*. *Trop. Med. Int. Health* 9(9):937–48
76. Grassmick RA, Rowley WA. 1973. Larval mortality of *Culex tarsalis* and *Aedes aegypti* when reared with different concentrations of *Tetrahymena pyriformis*. *J. Invertebr. Pathol.* 22(1):86–93
77. Gusmão DS, Santos AV, Marini DC, Bacci M Jr., Berbert-Molina MA, Lemos FJA. 2010. Culture-dependent and culture-independent characterization of microorganisms associated with *Aedes aegypti* (Diptera: Culicidae) (L.) and dynamics of bacterial colonization in the midgut. *Acta Trop.* 115(3):275–81
78. Habtewold T, Povelones M, Blagborough AM, Christophides GK. 2008. Transmission blocking immunity in the malaria non-vector mosquito *Anopheles quadriannulatus* species A. *PLOS Pathog.* 4(5):e1000070
79. Han YS, Thompson J, Kafatos FC, Barillas-Mury C. 2000. Molecular interactions between *Anopheles stephensi* midgut cells and *Plasmodium berghei*: the time bomb theory of ookinete invasion of mosquitoes. *EMBO J.* 19(22):6030–40
80. Hardy JL, Houk EJ, Kramer LD, Reeves WC. 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. *Annu. Rev. Entomol.* 28:229–62
81. Hauck ES, Antonova-Koch Y, Drexler A, Pietri J, Pakpour N, et al. 2013. Overexpression of phosphatase and tensin homolog improves fitness and decreases *Plasmodium falciparum* development in *Anopheles stephensi*. *Microbes Infect.* 15(12):775–87
82. Hegde S, Rasgon JL, Hughes GL. 2015. The microbiome modulates arbovirus transmission in mosquitoes. *Curr. Opin. Virol.* 15:97–102
83. Hillyer JF, Barreau C, Vernick KD. 2007. Efficiency of salivary gland invasion by malaria sporozoites is controlled by rapid sporozoite destruction in the mosquito haemocoel. *Int. J. Parasitol.* 37(6):673–81
84. Hillyer JF, Christensen BM. 2002. Characterization of hemocytes from the yellow fever mosquito, *Aedes aegypti*. *Histochem. Cell Biol.* 117(5):431–40
85. Hillyer JF, Christensen BM. 2005. Mosquito phenoloxidase and defensin colocalize in melanization innate immune responses. *J. Histochem. Cytochem.* 53(6):689–98
86. Hillyer JF, Estévez-Lao TY. 2010. Nitric oxide is an essential component of the hemocyte-mediated mosquito immune response against bacteria. *Dev. Comp. Immunol.* 34(2):141–49
87. Hillyer JF, Strand MR. 2014. Mosquito hemocyte-mediated immune responses. *Curr. Opin. Insect Sci.* 3:14–21

88. Hoa NT, Keene KM, Olson KE, Zheng L. 2003. Characterization of RNA interference in an *Anopheles gambiae* cell line. *Insect Biochem. Mol. Biol.* 33(9):949–57
89. Hopwood JA, Ahmed AM, Polwart A, Williams GT, Hurd H. 2001. Malaria-induced apoptosis in mosquito ovaries: a mechanism to control vector egg production. *J. Exp. Biol.* 204(16):2773–80
90. Horne KM, Vanlandingham DL. 2014. Bunyavirus-vector interactions. *Viruses* 6(11):4373–97
91. Horton AA, Wang B, Camp L, Price MS, Arshi A, et al. 2011. The mitogen-activated protein kinome from *Anopheles gambiae*: identification, phylogeny and functional characterization of the ERK, JNK and p38 map kinases. *BMC Genom.* 12(1):574
92. Hurd H, Grant KM, Arambage SC. 2006. Apoptosis-like death as a feature of malaria infection in mosquitoes. *Parasitology* 132 (Suppl. 1):S33–47
93. Hurd H, Taylor PJ, Adams D, Underhill A, Eggleston P. 2005. Evaluating the costs of mosquito resistance to malaria parasites. *Evolution* 59(12):2560–72
94. Joshi D, Pan X, McFadden MJ, Bevins D, Liang X, et al. 2017. The maternally inheritable *Wolbachia* *w*AlbB induces refractoriness to *Plasmodium berghei* in *Anopheles stephensi*. *Front. Microbiol.* 8:366
95. Jupatanakul N, Sim S, Dimopoulos G. 2014. The insect microbiome modulates vector competence for arboviruses. *Viruses* 6(11):4294–313
96. Keene KM, Foy BD, Sanchez-Vargas I, Beaty BJ, Blair CD, Olson KE. 2004. RNA interference acts as a natural antiviral response to O'nyong-nyong virus (*Alphavirus*; togaviridae) infection of *Anopheles gambiae*. *PNAS* 101(49):17240–45
97. Kelly EM, Moon DC, Bowers DF. 2012. Apoptosis in mosquito salivary glands: Sindbis virus-associated and tissue homeostasis. *J. Gen. Virol.* 93(11):2419–24
98. Kerwin JL. 2007. Oomycetes: *Lagenidium giganteum*. *J. Am. Mosq. Control Assoc.* 23(Suppl. 2):50–57
99. Kim W, Koo H, Richman AM, Seeley D, Vizioli J, et al. 2004. Ectopic expression of a cecropin transgene in the human malaria vector mosquito *Anopheles gambiae* (Diptera: Culicidae): effects on susceptibility to *Plasmodium*. *J. Med. Entomol.* 41(3):447–55
100. King JG, Hillyer JF. 2012. Infection-induced interaction between the mosquito circulatory and immune systems. *PLOS Pathog.* 8(11):e1003058
101. King JG, Hillyer JF. 2013. Spatial and temporal in vivo analysis of circulating and sessile immune cells in mosquitoes: hemocyte mitosis following infection. *BMC Biol.* 11:55
102. Kittayapong P, Baisley KJ, O'Neill SL. 1999. A mosquito densovirus infecting *Aedes aegypti* and *Aedes albopictus* from Thailand. *Am. J. Trop. Med. Hyg.* 61(4):612–17
103. Kokoza V, Ahmed A, Woon Shin S, Okafor N, Zou Z, Raikhel AS. 2010. Blocking of *Plasmodium* transmission by cooperative action of Cecropin A and Defensin A in transgenic *Aedes aegypti* mosquitoes. *PNAS* 107(18):8111–16
104. Kumar S, Gupta L, Han YS, Barillas-Mury C. 2004. Inducible peroxidases mediate nitration of *Anopheles* midgut cells undergoing apoptosis in response to *Plasmodium* invasion. *J. Biol. Chem.* 279(51):53475–82
105. Lai S-C, Chen C-C, Hou RF. 2002. Immunolocalization of prophenoloxidase in the process of wound healing in the mosquito *Armigeres subalbatus* (Diptera: Culicidae). *J. Med. Entomol.* 39(2):266–74
106. Levashina EA, Moita LF, Blandin S, Vriend G, Lagueux M, Kafatos FC. 2001. Conserved role of a complement-like protein in phagocytosis revealed by dsRNA knockout in cultured cells of the mosquito, *Anopheles gambiae*. *Cell* 104(5):709–18
107. Linley JR, Nielsen HT. 1968. Transmission of a mosquito iridescent virus in *Aedes taeniorhynchus*: I. Laboratory experiments. *J. Invertebr. Pathol.* 12(1):7–16
108. Liu B, Behura SK, Clem RJ, Schneemann A, Becnel J, et al. 2013. P53-mediated rapid induction of apoptosis conveys resistance to viral infection in *Drosophila melanogaster*. *PLOS Pathog.* 9(2):e1003137
109. Lombardo F, Christophides GK. 2016. Novel factors of *Anopheles gambiae* haemocyte immune response to *Plasmodium berghei* infection. *Parasites Vectors* 9:78
110. Lombardo F, Ghani Y, Kafatos FC, Christophides GK. 2013. Comprehensive genetic dissection of the hemocyte immune response in the malaria mosquito *Anopheles gambiae*. *PLOS Pathog.* 9(1):e1003145
111. Lowenberger CA, Ferdig MT, Bulet P, Khalili S, Hoffmann JA, Christensen BM. 1996. *Aedes aegypti*: induced antibacterial proteins reduce the establishment and development of *Brugia malayi*. *Exp. Parasitol.* 83(2):191–201

112. Lowenberger CA, Kamal S, Chiles J, Paskewitz S, Bulet P, et al. 1999. Mosquito–*Plasmodium* interactions in response to immune activation of the vector. *Exp. Parasitol.* 91(1):59–69
113. Lowenberger CA, Smartt CT, Bulet P, Ferdig MT, Severson DW, et al. 1999. Insect immunity: molecular cloning, expression, and characterization of cDNAs and genomic DNA encoding three isoforms of insect defensin in *Aedes aegypti*. *Insect Mol. Biol*. 8(1):107–18
114. Michel K, Suwanchaichinda C, Morlais I, Lambrechts L, Cohuet A, et al. 2006. Increased melanizing activity in *Anopheles gambiae* does not affect development of *Plasmodium falciparum*. *PNAS* 103(45):16858–63
115. Miesen P, Joosten J, van Rij RP. 2016. PIWIs go viral: arbovirus-derived piRNAs in vector mosquitoes. *PLOS Pathog*. 12(12):e1006017
116. Moita LF, Wang-Sattler R, Michel K, Zimmermann T, Blandin S, et al. 2005. In vivo identification of novel regulators and conserved pathways of phagocytosis in *A. gambiae*. *Immunity* 23(1):65–73
117. Molina-Cruz A, Garver LS, Alabaster A, Bangiolo L, Haile A, et al. 2013. The human malaria parasite *Pfs47* gene mediates evasion of the mosquito immune system. *Science* 340(6135):984–87
118. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu GJ, Pyke AT, et al. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* 139(7):1268–78
119. Müller H-M, Dimopoulos G, Blass C, Kafatos FC. 1999. A hemocyte-like cell line established from the malaria vector *Anopheles gambiae* expresses six prophenoloxidase genes. *J. Biol. Chem.* 274(17):11727–35
120. Muturi EJ, Bara JJ, Rooney AP, Hansen AK. 2016. Midgut fungal and bacterial microbiota of *Aedes triseriatus* and *Aedes japonicus* shift in response to La Crosse virus infection. *Mol. Ecol.* 25(16):4075–90
121. Myles KM, Wiley MR, Morazzani EM, Adelman ZN. 2008. Alphavirus-derived small RNAs modulate pathogenesis in disease vector mosquitoes. *PNAS* 105(50):19938–43
122. Nappi AJ, Christensen BM. 2005. Melanogenesis and associated cytotoxic reactions: applications to insect innate immunity. *Insect Biochem. Mol. Biol.* 35(5):443–59
123. Nappi AJ, Poirié M, Carton Y. 2009. The role of melanization and cytotoxic by-products in the cellular immune responses of *Drosophila* against parasitic wasps. *Adv. Parasitol.* 70:99–121
124. Neafsey DE, Waterhouse RM, Abai MR, Aganezov SS, Alekseyev MA, et al. 2015. Mosquito genomics. Highly evolvable malaria vectors: the genomes of 16 *Anopheles* mosquitoes. *Science* 347(6217):1258522
125. Neira Oviedo M, VanEkeris L, Corena-Mcleod MDP, Linser PJ. 2008. A microarray-based analysis of transcriptional compartmentalization in the alimentary canal of *Anopheles gambiae* (Diptera: Culicidae) larvae. *Insect Mol. Biol.* 17(1):61–72
126. Ocampo CB, Caicedo PA, Jaramillo G, Ursic Bedoya R, Baron O, et al. 2013. Differential expression of apoptosis related genes in selected strains of *Aedes aegypti* with different susceptibilities to dengue virus. *PLOS ONE* 8(4):e61187
127. Oliveira GdA, Lieberman J, Barillas-Mury C. 2012. Epithelial nitration by a peroxidase/NOX5 system mediates mosquito antiplasmodial immunity. *Science* 335(6070):856–59
128. Olson KE, Blair CD. 2015. Arbovirus–mosquito interactions: RNAi pathway. *Curr. Opin. Virol.* 15:119–26
129. Orfano AS, Nacif-Pimenta R, Duarte APM, Villegas LM, Rodrigues NB, et al. 2016. Species-specific escape of *Plasmodium* sporozoites from oocysts of avian, rodent, and human malarial parasites. *Malar. J.* 15(1):394
130. Osta MA, Christophides GK, Kafatos FC. 2004. Effects of mosquito genes on plasmodium development. *Science* 303(5666):2030–32
131. Paily KP, Balaraman K. 2000. Susceptibility of ten species of mosquito larvae to the parasitic nematode *Romanomermis iyengari* and its development. *Med. Vet. Entomol.* 14(4):426–29
132. Palmer CA, Wittrock DD, Christensen BM. 1986. Ultrastructure of Malpighian tubules of *Aedes aegypti* infected with *Dirofilaria immitis*. *J. Invertebr. Pathol*. 48(3):310–17
133. Pan X, Zhou G, Wu J, Bian G, Lu P, et al. 2012. *Wolbachia* induces reactive oxygen species (ROS)-dependent activation of the Toll pathway to control dengue virus in the mosquito *Aedes aegypti*. *PNAS* 109(1):E23–31
134. Parikh GR, Oliver JD, Bartholomay LC. 2009. A haemocyte tropism for an arbovirus. *J. Gen. Virol.* 90(2):292–96

135. Pinto SB, Kafatos FC, Michel K. 2008. The parasite invasion marker SRPN6 reduces sporozoite numbers in salivary glands of *Anopheles gambiae*. *Cell. Microbiol.* 10(4):891–98
136. Pinto SB, Lombardo F, Koutsos AC, Waterhouse RM, McKay K, et al. 2009. Discovery of plasmodium modulators by genome-wide analysis of circulating hemocytes in *Anopheles gambiae*. *PNAS* 106(50):21270–75
137. Platzer EG. 2007. Mermithid nematodes. *J. Am. Mosq. Control Assoc.* 23:58–64
138. Porter CH, DeFoliart GR. 1985. Gonotrophic age, insemination, and *Ascogregarina* infection in a southern Wisconsin population of *Aedes triseriatus*. *J. Am. Mosq. Control Assoc.* 1(2):238–40
139. Povelones M, Bhagavatula L, Yassine H, Tan LA, Upton LM, et al. 2013. The CLIP-domain serine protease homolog SPCLIP1 regulates complement recruitment to microbial surfaces in the malaria mosquito *Anopheles gambiae*. *PLOS Pathog.* 9(9):e1003623
140. Povelones M, Waterhouse RM, Kafatos FC, Christophides GK. 2009. Leucine-rich repeat protein complex activates mosquito complement in defense against plasmodium parasites. *Science* 324(5924):258–61
141. Powers AM, Olson KE, Higgs S, Carlson JO, Beaty BJ. 1994. Intracellular immunization of mosquito cells to LaCrosse virus using a recombinant Sindbis virus vector. *Virus Res.* 32(1):57–67
142. Ramirez JL, Souza-Neto J, Torres Cosme R, Rovira J, Ortiz A, et al. 2012. Reciprocal tripartite interactions between the *Aedes aegypti* midgut microbiota, innate immune system and dengue virus influences vector competence. *PLOS Negl. Trop. Dis.* 6(3):e1561
143. Richman AM, Bulet P, Hetru C, Barillas-Mury C, Hoffmann JA, Kafalos FC. 1996. Inducible immune factors of the vector mosquito *Anopheles gambiae*: biochemical purification of a defensin antibacterial peptide and molecular cloning of preprodefensin cDNA. *Insect Mol. Biol.* 5(3):203–10
144. Riehle MM, Xu J, Lazzaro BP, Rottschaefer SM, Coulibaly B, et al. 2008. *Anopheles gambiae* APL1 is a family of variable LRR proteins required for Rel1-mediated protection from the malaria parasite, *Plasmodium berghei*. *PLOS ONE* 3(11):e3672
145. Rodrigues J, Brayner FA, Alves LC, Dixit R, Barillas-Mury C. 2010. Hemocyte differentiation mediates innate immune memory in *Anopheles gambiae* mosquitoes. *Science* 329(5997):1353–55
146. Saiyasombat R, Bolling BG, Brault AC, Bartholomay LC, Blitvich BJ. 2011. Evidence of efficient transovarial transmission of Culex flavivirus by *Culex pipiens* (Diptera: Culicidae). *J. Med. Entomol.* 48(5):1031–38
147. Sang RC, Gichogo A, Gachoya J, Dunster MD, Ofula V, et al. 2003. Isolation of a new flavivirus related to Cell fusing agent virus (CFAV) from field-collected flood-water *Aedes* mosquitoes sampled from a dambo in central Kenya. *Arch. Virol.* 148(6):1085–93
148. Scholte E-J, Knols BGJ, Samson RA, Takken W. 2004. Entomopathogenic fungi for mosquito control: a review. *J. Insect Sci.* 4:19
149. Shahabuddin M, Fields I, Bulet P, Hoffmann JA, Miller LH. 1998. *Plasmodium gallinaceum*: differential killing of some mosquito stages of the parasite by insect defensin. *Exp. Parasitol.* 89(1):103–12
150. Shaw WR, Marcenac P, Childs LM, Buckee CO, Baldini F, et al. 2016. *Wolbachia* infections in natural *Anopheles* populations affect egg laying and negatively correlate with *Plasmodium* development. *Nat. Commun.* 7:11772
151. Shelly S, Lukinova N, Bambina S, Berman A, Cherry S. 2009. Autophagy is an essential component of *Drosophila* immunity against vesicular stomatitis virus. *Immunity* 30(4):588–98
152. Shin SW, Bian GW, Raikhel AS. 2006. A Toll receptor and a cytokine, Toll5a and Spz1c, are involved in Toll antifungal immune signaling in the mosquito *Aedes aegypti*. *J. Biol. Chem.* 281(51):39388–95
153. Shin SW, Kokoza V, Bian G, Cheon H-M, Kim YJ, Raikhel AS. 2005. REL1, a homologue of *Drosophila dorsal*, regulates Toll antifungal immune pathway in the female mosquito *Aedes aegypti*. *J. Biol. Chem.* 280(16):16499–507
154. Shoulkamy MA, Abdelzaher HMA, Shahin AAB. 2001. Ultrastructural changes in the muscles, midgut, hemopoietic organ, imaginal discs and malpighian tubules of the mosquito *Aedes aegypti* larvae infected by the fungus *Coelomomyces stegomyiae*. *Mycopathologia* 149(2):99–106
155. Sinden RE, Billingsley PF. 2001. *Plasmodium* invasion of mosquito cells: hawk or dove? *Trends Parasitol.* 17(5):209–12

156. Thangamani S, Huang J, Hart CE, Guzman H, Tesh RB. 2016. Vertical transmission of Zika virus in *Aedes aegypti* mosquitoes. *Am. J. Trop. Med. Hyg.* 95(5):1169–73
157. Vaidyanathan R, Scott TW. 2006. Apoptosis in mosquito midgut epithelia associated with West Nile virus infection. *Apoptosis* 11(9):1643–51
158. Valero-Jiménez CA, Debets AJM, van Kan JAL, Schoustra SE, Takken W, et al. 2014. Natural variation in virulence of the entomopathogenic fungus *Beauveria bassiana* against malaria mosquitoes. *Malar. J.* 13(1):479
159. Vasilakis N, Tesh RB. 2015. Insect-specific viruses and their potential impact on arbovirus transmission. *Curr. Opin. Virol.* 15:69–74
160. Vernick KDD, Fujioka H, Seeley DCC, Tandler B, Aikawa M, Miller LHH. 1995. *Plasmodium gallinaceum*: a refractory mechanism of ookinete killing in the mosquito, *Anopheles gambiae*. *Exp. Parasitol.* 80(4):583–95
161. Vlachou D, Schlegelmilch T, Christophides GK, Kafatos FC. 2005. Functional genomic analysis of midgut epithelial responses in *Anopheles* during plasmodium invasion. *Curr. Biol.* 15(13):1185–95
162. Vlachou D, Zimmermann T, Cantera R, Janse CJ, Waters AP, Kafatos FC. 2004. Real-time, in vivo analysis of malaria ookinete locomotion and mosquito midgut invasion. *Cell. Microbiol.* 6(7):671–85
163. Wang H, Gort T, Boyle DL, Clem RJ. 2012. Effects of manipulating apoptosis on Sindbis virus infection of *Aedes aegypti* mosquitoes. *J. Virol.* 86(12):6546–54
164. Wang Y-H, Hu Y, Xing L-S, Jiang H, Hu S-N, et al. 2015. A critical role for CLSP2 in the modulation of antifungal immune response in mosquitoes. *PLOS Pathog.* 11(6):e1004931
165. Ward TW, Jenkins MS, Afanasiev BN, Edwards M, Duda BA, et al. 2001. *Aedes aegypti* transducing densovirus pathogenesis and expression in *Aedes aegypti* and *Anopheles gambiae* larvae. *Insect Mol. Biol.* 10(5):397–405
166. Warr E, Aguilar R, Dong YM, Mahairaki V, Dimopoulos G. 2007. Spatial and sex-specific dissection of the *Anopheles gambiae* midgut transcriptome. *BMC Genom.* 8:37
167. Washburn JO, Anderson JR, Mercer DR. 1989. Emergence characteristics of *Aedes sierrensis* (Diptera: Culicidae) from California treeholes with particular reference to parasite loads. *J. Med. Entomol.* 26(3):173–82
168. Waterhouse RM, Kriventseva EV, Meister S, Xi ZY, Alvarez KS, et al. 2007. Evolutionary dynamics of immune-related genes and pathways in disease-vector mosquitoes. *Science* 316(5832):1738–43
169. Watts DM, Pantuwatana S, Defoliart GR, Yuill TM, Thompson WH. 1973. Transovarial transmission of LaCrosse virus (California encephalitis group) in the mosquito, *Aedes triseriatus*. *Science* 182(4117):1140–41
170. Weaver SC, Scott TW, Lorenz LH, Lerdthusnee K, Romoser WS. 1988. Togavirus-associated pathologic changes in the midgut of a natural mosquito vector. *J. Virol.* 62(6):2083–90
171. Whitten MMA, Shiao SH, Levashina EA. 2006. Mosquito midguts and malaria: cell biology, compartmentalization and immunology. *Parasite Immunol.* 28(4):121–30
172. Xu X, Dong Y, Abraham EG, Kocan A, Srinivasan P, et al. 2005. Transcriptome analysis of *Anopheles stephensi–Plasmodium berghei* interactions. *Mol. Biochem. Parasitol.* 142(1):76–87
173. Yassine H, Kamareddine L, Chamat S, Christophides GK, Osta MA. 2014. A serine protease homolog negatively regulates TEP1 consumption in systemic infections of the malaria vector *Anopheles gambiae*. *J. Innate Immun.* 6(6):806–18
174. Yassine H, Kamareddine L, Osta MA. 2012. The mosquito melanization response is implicated in defense against the entomopathogenic fungus *Beauveria bassiana*. *PLOS Pathog.* 8(11):e1003029
175. Zhang Q, Hua G, Adang MJ. 2017. Effects and mechanisms of *Bacillus thuringiensis* crystal toxins for mosquito larvae. *Insect Sci.* 24(5):714–29
176. Zouache K, Michelland RJ, Failloux AB, Grundmann GL, Mavingui P. 2012. Chikungunya virus impacts the diversity of symbiotic bacteria in mosquito vector. *Mol. Ecol.* 21(9):2297–309