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Role of the Vector in Arbovirus Transmission

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Abstract

Many arboviral diseases are uncontrolled, and the viruses that cause them are globally emerging or reemerging pathogens that produce significant disease throughout the world. The increased spread and prevalence of disease are occurring during a period of substantial scientific growth in the vectorborne disease research community. This growth has been supported by advances in genomics and proteomics, and by the ability to genetically alter disease vectors. For the first time, researchers are elucidating the molecular details of vector host-seeking behavior, the susceptibility of disease vectors to arboviruses, the immunological control of infection in disease vectors, and the determinants that facilitate transmission of arboviruses from a vector to a host. These discoveries are facilitating the development of novel strategies to combat arboviral disease, including the release of transgenic mosquitoes harboring dominant lethal genes, the introduction of arbovirus-blocking microbes into mosquito populations, and the development of acquisition- and transmission-blocking therapeutics. Understanding the role of the vector in arbovirus transmission has provided critical practical and theoretical tools to control arboviral disease.

Keywords

mosquito; host; immune response; saliva protein

INTRODUCTION

Arboviruses are defined as viruses that are transmitted to a mammalian host by an arthropod vector. In humans, relevant disease-spreading arthropods include mosquitoes and ticks, among others. This enzootic transmission cycle requires that virus, vector, and host spatially and temporally interact in a way that facilitates acquisition of a virus from an infected host

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into a susceptible vector, dissemination of the virus throughout the vector to the salivary glands, and transmission of the virus to a host. Numerous complex factors play a role in this dynamic relationship, including vector capacity and host susceptibility. Vector capacity describes all aspects of a vector's ability to acquire, maintain, and transmit a pathogen, including the feeding habits and life span of the vector species (1). Host susceptibility includes a complex interplay of genetic and immunological determinants.

Currently, the Centers for Disease Control and Prevention's list of arboviruses and related zoonotic viruses encompasses more than 600 known arboviruses. Over 80 of these are known human pathogens. Our global survey of arboviruses is likely incomplete, and ongoing surveillance is necessary to understand how viruses spill over into the human population. Many arboviruses have evolved the ability to infect both arthropod and mammalian hosts, leading to widespread human infection and disease. Understanding the role of the vector in arbovirus transmission is critical to the development of novel strategies to control the spread of disease. Here we review features of vector mosquitoes that influence arbovirus transmission. We compile work from diverse research fields and identify novel targets for rational therapeutic and vaccine design based on vector rather than viral or host components.

DENGUE AND WEST NILE VIRUSES

Currently, dengue virus (DENV) is the most problematic arbovirus to the human population; globally, it infects 100–390 million individuals, causing up to 96 million symptomatic infections and leading to 12,500 deaths per year—mostly among young children (2, 3). DENV is considered a reemerging pathogen largely because of the increasing range of *Aedes aegypti* and *Aedes albopictus* vectors. These mosquito species are spreading around the world due to globalization, a warming climate, and other factors (4). Epidemics of DENV have primarily been restricted to resource-limited areas of the world, regions that include 2.5 billion individuals or 40% of the world population, making DENV a significant burden on these struggling economies (3).

Unpredictable outbreaks of arboviral disease, such as the introduction of West Nile virus (WNV) into the United States in 1999, highlight the need to control the spread of arboviruses and their disease vectors. WNV has become endemic to the Unites States and is responsible for unpredictable epidemics that result in hundreds to thousands of reported cases of neuroinvasive diseases each year (5). Dozens to a few hundred deaths occur each year from disease caused by WNV infection (5). Since the introduction of WNV in the United States, over 3 million people have been infected (6). The reason for the variability in epidemic severity is unclear, but it may be in part due to interactions between the vector mosquito and its environment, including temperature changes and availability of preferred hosts (7). There are no specific treatments or prophylactics available for either DENV or WNV, and treatments are limited for many other human disease-causing arboviruses.

MOSQUITO VECTORS

A number of mosquito species serve as vectors of arboviral disease in nature, and many more are competent vectors in a laboratory setting. We can expect fluctuations in time of the

predominant species involved in arbovirus transmission. At present, *Aedes* and *Culex* spp. are the major vectors of medically important arboviral diseases in humans (Table 1).

Aedes aegypti and *Aedes albopictus*

Aedes aegypti is a tropical mosquito that has recently become endemic to many novel geographic locations due to globalization, a warming climate, and the disuse of DDT (dichlorodiphenyltrichloroethane) as an insecticide. *Aedes aegypti* is the primary vector of DENV, chikungunya virus (CHIKV), and yellow fever virus (YFV) and has colonized southern regions of the United States including parts of Florida, California, and Texas (Table 1) (8, 9). *Aedes albopictus* is also a competent vector for many arboviruses including DENV and CHIKV, although it typically leads to milder epidemics than *Aedes aegypti* does (10). *Aedes albopictus* has also been found in nature infected with WNV, eastern equine encephalitis virus (EEEV), and Japanese encephalitis virus (JEV)(11).

Aedes albopictus is a temperate mosquito that has colonized a major portion of the United States. In the north, *Aedes albopictus* has become endemic from New Jersey to the Midwest. In the south, it has become endemic from Florida to Texas (10). It does not appear that *Aedes albopictus* can overwinter north of Chicago; however, genetic and climatic variation can lead to enhanced survival of mosquito eggs during winter conditions (12–14).

At this point, it is unclear whether DENV can become established in the United States *Aedes albopictus* population (15). However, studies suggest that *Aedes albopictus* can maintain a pool of DENV that is utilized by *Aedes aegypti* (10). This possibility suggests that DENV can move between these two species. Currently, they overlap in multiple regions throughout the southern United States. Interestingly, when inhabiting the same geographical region, *Aedes albopictus* tends to displace *Aedes aegypti* from competing environments due to the one-directional insemination of *Aedes aegypti* females by *Aedes albopictus* males (16, 17). It should be determined whether the competitive advantage of *Aedes albopictus* over *Aedes aegypti* can lead to the selection of DENV variants that are more effectively transmitted by *Aedes albopictus*. This scenario would facilitate the dissemination of DENV into the United States and other more temperate regions that are refractory to colonization by *Aedes aegypti*.

Culex Species

Several species of *Culex* have the ability to serve as vectors of arboviruses such as WNV, JEV, and St. Louis encephalitis virus (SLEV) (Table 1). Though *Culex* spp. typically obtain their blood meals from birds instead of mammals, their ability to harbor and transmit human pathogens during the occasional human blood meal can lead to severe and potentially fatal disease (18). WNV is maintained in an enzootic transmission cycle between *Culex* spp. and avian hosts. Humans, horses, and other animals are considered dead-end hosts and are usually targeted by *Culex* spp. when the preferred avian host is not available (18, 19). Avian hosts may not be available due to changes in climate or migration patterns, leading to increased human exposure to infected mosquitoes (18). The most important *Culex* spp. in terms of arbovirus exposure and human infection vary depending on the geographical region and may be subject to change depending on climate and host availability. Generally, *Culex*

pipiens, Culex tarsalis, and *Culex quinquefasciatus* are responsible for transmission of WNV in the United States. Different *Culex* spp. have been implicated in disease transmission in other countries.

VIRUS-VECTOR INTERACTIONS

Virus Acquisition

Mosquitoes become infected with arboviruses during feeding on an infected host. Virus travels into the mosquito midgut along with the blood meal. In order for the mosquito to acquire an arboviral infection, it is essential that the arbovirus has evolved a mechanism to breach the midgut barrier. This consists of both immunological and physical barriers including proteolytic enzyme upregulation, the RNA interference (RNAi) pathway, peritrophic matrix formation, antimicrobial molecule influx, and the physical barrier of midgut epithelial cells (20–22). The presence of normal bacterial flora in the insect midgut also negatively influences arbovirus acquisition. This antiviral state may be due to the production of reactive oxygen species by the vector to control bacterial growth, or perhaps to competition for metabolic resources (23).

It is largely unknown how arboviruses evade the midgut barrier, although incoming arboviral populations do undergo a genetic bottleneck (24). Presumably, certain genotypes are maladapted to the midgut environment, and a strong selective pressure is exerted (25). If this is the case, it is not clear what phenotype the selected population has that makes it well adapted to the mosquito midgut environment. It is also possible that a nonselective reduction in the viral population leads to the genetic bottleneck and that infection of midgut epithelial cells simply requires a high titer of virus in the blood meal.

Vector Response to Infection

The acquisition of an arbovirus leads to transcriptomic and proteomic alterations in mosquito vectors. Many studies have been performed to evaluate the effect of arbovirus infection on mosquito gene expression, protein levels, and immune system responses, as well as the impacts these changes have on the vector's life cycle (26–28). For many genes, it is unclear whether altered gene and protein expression is directed by the virus or the vector. Generally, microarray analysis has shown that flavivirus infection leads to the up regulation of many genes in *Aedes aegypti*, including transcription factors, ion-binding proteins, and many metabolic proteins, and leads to the downregulation of protease and pupal cuticle protein genes, among others (26). Transcriptomic analysis of *Culex* spp. during infection has revealed that many genes related to transport and metabolism are upregulated upon infection with WNV (29). The immune response of both *Culex* and *Aedes* spp. to arbovirus infection includes the RNAi pathway, the JAK-STAT pathway, and Toll signaling (30–32). Immune responses may also be triggered by factors in the blood such as insulin, which may stimulate the ERK pathway and lead to a broad antiviral response (33). It is likely that viral infection directs some alteration in gene expression and immune function in the mosquito, and these genes and proteins may represent ideal targets for blocking arbovirus infections in the vector.

Dissemination to the Salivary Glands

For those arbovirus genotypes that do survive the midgut barrier, it takes several days to disseminate to distal tissues including the salivary gland (34, 35). It remains to be seen whether arboviruses undergo further selective pressure in the hemolymph or other distal tissues. However, after the midgut, a second bottleneck has been observed in the salivary glands, suggesting either that certain viral genotypes are maladapted to infect this organ or that a strong nonselective reduction in the viral population occurs at that site (24). While mutations can become fixed in viral populations after passage in insect cell culture or live mosquitoes, it is clear that alternating between vector and host constrains the rate of evolutionary change (36–38). These constraints may limit the interaction of viral and cellular components to evolutionarily conserved molecules that are present in both vector and host. However, selection and accumulation of vector-specific mutations may occur in a single round of infection. These mutations may impact transmission or pathogenesis in the host. For example, research on DENV replication kinetics in Aedes aegypti has shown that virus isolates that are more commonly associated with dengue hemorrhagic fever epidemics can outcompete virus isolates associated with dengue fever (39).

VIRUS-VECTOR-HOST INTERACTIONS

Establishment of an Enzootic Transmission Cycle

Mosquitoes as we know them have been on this planet for at least 50 million years (40). An increasing number of arboviruses have been classified as infecting only arthropods, raising the questions of where these viruses came from and which genetic changes were required for adaptation to their new arthropod hosts (41, 42). These viruses may have been introduced into arthropods from the environment or during a sugar or blood feeding event. Other arboviruses can infect both arthropods and mammals. Unknown genetic changes are required for the adaptation of arboviruses to mammalian hosts. At a minimum, the establishment of an enzootic transmission cycle would require the selection of viral proteins that can interact with diverse molecules including cell surface receptors and entry factors, immune components, protein translation machinery, and protein export machinery in both arthropod and mammalian cells. It is reasonable to hypothesize that many individual mutations are required for an arthropod-only virus to transition to an enzootic transmission cycle that includes a mammalian host. It is unclear how the selective environment in disease vectors influences this process, and it is possible that certain arthropods or environments more effectively select for viruses that can be transmitted to a mammalian host. Understanding how this selective process leads to the evolution of pathogenic arboviruses is critical to control emerging arboviral diseases.

Factors Influencing Host Seeking

Mosquito species feed on either plant nectar, vertebrate blood, or both plant nectar and vertebrate blood (43–46). Mosquitoes use different visual, chemical, and sensory cues to seek out nectar and blood meals (47,48). Disease vectors sense various attractive cues to host seek including movement, body heat, CO_2, and volatile compounds released from host skin and normal bacterial flora (48–52). Discrimination of hosts can be further stratified at the genus level. For instance, *Culex* spp. prefer to feed on American robins in some locations

and will feed on humans only if their preferred avian host is not available (18, 19). The genetic alterations required for host discrimination are largely unknown, although it is clear that insects detect attractive cues through several molecules including odorant receptors and an obligate coreceptor called orco (48). Importantly, genetic disruption of *Aedes aegypti* orco protein led to reduced discrimination between animal and human scent in the presence of CO_2 and to reduced attraction to honey and human scent in the absence of CO_2 (48). Detection of skin odor has been mapped to CO_2-sensitive olfactory neurons, which suggests that orco is expressed in this cell type (53). Detection of CO_2 appears to amplify scent signals in the mosquito. These data confirm that molecular evolution is key to host detection and discrimination.

Influence of Saliva on Transmission

When infected mosquitoes probe host skin for a source of blood, they inoculate virus-infected saliva mostly into extravascular spaces in the dermis (54–56). The majority of in vivo arbovirus research uses laboratory techniques such as needle inoculation of virus that may alter or miss elements of the natural infectious process, which is typically modified by vector saliva (57). Accordingly, multiple reports have identified a role for mosquito saliva in the modulation of arbovirus infectivity and transmission both in vitro and in vivo (Table 2) (58–67).

Although a correlation between saliva-mediated infectivity enhancement and the modulation of interferon (IFN), tumor necrosis factor (TNF), and T helper 1/2 (Th1/Th2) immune responses has been shown, no study has directly tested whether modulation of the immune response is required for saliva-mediated infectivity enhancement or is just a consequence of exposure to saliva allergens (57, 59, 61, 62, 68). That said, most studies do show that saliva can suppress IFN expression in both in vitro and in vivo model systems (62, 69–74). Suppression of the host innate immune response would be expected to have an impact on virus transmission; however, there are no conclusive experimental data that implicate a specific saliva factor in immunomodulation. Further, saliva-mediated enhancement of DENV infectivity occurs in cells and mice lacking the type I IFN response, suggesting that enhancement in this context is not the result of modulation of the innate immune system (63). The role of the immune response in saliva-mediated infectivity enhancement is not clear. It is clear, however, that both mosquito saliva and virus must be inoculated at the same cutaneous site for infectivity enhancement to occur (61, 75), suggesting that a mosquito saliva component alters the local inoculation site in favor of virus transmission.

Complex Nature of Mosquito Saliva

Salivary gland transcriptomes (sialotranscriptomes) have been generated for multiple mosquito species, and these data suggest that over 100 proteins are expressed and secreted into saliva (76, 77). All hematophagous arthropods appear to express saliva proteins with antiplatelet, anticoagulation, and vasodilation activities, and many of these proteins have been characterized genetically and biochemically (76). There are many other proteins whose role in blood feeding and virus transmission remains largely uncharacterized, including D7 proteins, odorant-binding proteins, antimicrobial proteins, serpins, nucleotidases, serine proteases, lectins, mucins, and various other antigens of unknown homology and function

(76). It will be important to determine whether any of these proteins play a role in arbovirus transmission.

Due to the complex nature of mosquito saliva, certain saliva factors may inhibit and others may enhance virus infectivity (63). In fact, a cecropin-like peptide that is expressed in the salivary glands of *Aedes aegypti* has been shown to lower DENV infectivity (28). Additionally, immunization with recombinant D7 protein, one of the most immunogenic proteins in saliva, led to enhanced WNV infection in mice, suggesting that the protein itself is directly or indirectly inhibitory to infection (78). A recombinant pupal cuticle protein that is expressed in *Aedes aegypti* salivary glands was also able to inhibit WNV infection in an encephalitic mouse model of infection (26). Interestingly, we found that certain fractions of high-performance liquid chromatography (HPLC)-fractionated *Aedes aegypti* salivary gland extract (SGE) increased and others decreased DENV infectivity in vitro, whereas transmission enhancement was observed using nonfractionated SGE in vivo (63). This suggested that enhancing factors may be dominant over inhibiting factors in vivo. The vast majority of studies using coinoculation of virus with SGE or live mosquitoes to deliver a virus inoculum suggest that whole saliva enhances rather than inhibits infectivity (Table 2).

Saliva-Induced Allergic Response and Transmission

Mosquito saliva contains potent allergens. The bite of a mosquito and subsequent injection of saliva into human skin almost always trigger an allergic reaction. Treatment of skin with irritants or allergens modulates the dermal environment and induces the migration of Langerhans cells to draining lymph nodes (79). Although the mechanistic details of how irritants and allergens induce Langerhans cell migration are not fully defined, it is correlated with the breakdown of integrinmediated interactions with the extracellular matrix and a fibroblast and interleukin 10 (IL-10)-dependent switch of Langerhans cells to a macrophage-like phenotype (80, 81). Many allergens such as dust mite Der p proteins, *Aspergillus* spp. Asp proteins, certain pollens, and cockroach proteins are proteases that cleave tight-junction molecules and activate PAR2 (82). This results in increased epithelial permeability and production of chemokines. These proteases also cleave components of complement, CD40, CD25, and CD23, leading to various cellular effects ranging from recruiting innate immune cells to stimulating immunoglobulin E (IgE) production by B cells (82). Protease allergens also elicit a Th2 response, which has been suggested as the cause of saliva-mediated infectivity enhancement (59, 68, 83).

We identified that serine protease activity in mosquito saliva is responsible for transmission enhancement in vivo (63). Our mechanistic studies suggested that the salivary serine protease breaks down the extracellular matrix laid down by interstitial fibroblasts, which may lead to increased cell migration at the inoculation site. Blocking extracellular matrix breakdown with a chemical inhibitor completely inhibited saliva-mediated enhancement of viral RNA in draining lymph nodes (Figure 1) (63). Langerhans cells are targets of DENV infection in vivo (84), whereas macrophages may serve to control infection at this early time point (85). Given this context, we hypothesize that a mosquito saliva serine protease, like known protease allergens, disrupts the barrier function of skin and induces Langerhans cell migration to draining lymph nodes. Induction of Langerhans cell migration would increase

the probability of interaction with immobilized virions and dissemination to distal sites in the host (Figure 1).

TARGETING THE VECTOR: THE FUTURE OF ACQUISITION-AND TRANSMISSION-BLOCKING TECHNOLOGIES

The development of therapeutics that target either a pathogen or vector protein to prevent transmission to human hosts is essential to the eradication of many vector-borne diseases. Acquisitionblocking vaccines (ABVs) are currently being developed and have been successful at preventing malaria infection of *Anopheles* mosquitoes (86–88). One of these, an ABV developed against the *Plasmodium* protein Pfs25, was able to prevent the acquisition of malaria from infected mice by naive mosquitoes (86). Another group found that vaccinating mice with the mosquito protein serpin-2 prevented the acquisition of *Plasmodium berghei* by a naive group of mosquitoes (89). In addition, an arthropod-specific transmission-blocking vaccine (TBV) based on the outer surface protein A (OspA) of Borrelia burgdorferi, the causative agent of Lyme disease, has been shown to protect mice from spirochete infection. Proteins from sand fly saliva have also been used successfully as TBVs to prevent the transmission of *Leishmania* (90–92). The studies above suggest that it is theoretically possible to use mosquito proteins as TBVs to prevent the transmission of DENV and other arboviruses.

Advances in techniques to biochemically, genetically, and physically manipulate mosquito vectors have resulted in an explosion of novel vector control strategies to combat arboviral disease. For instance, the adaptation of RNAi and transgenic techniques used in *Drosophila* and other species has facilitated experimentation with gene-altered mosquitoes. Genes can now be upregulated, downregulated, knocked in, and knocked out of disease-causing mosquitoes. Further, the annotation of disease vector genomes has facilitated the use of high-throughput technologies such as RNA Seq and proteomics. These technologies will become more refined in the near future, leading to tissue-targeted gene modulation and a global collection of transgenic mosquito colonies with various genetic alterations, as well as a detailed understanding of the molecular determinants that govern virus transmission. Novel strategies are already being developed and tested that could provide valuable tools to reduce arboviral disease (Figure 2). The following sections detail exciting progress in the development of various vector-based control measures and therapeutics.

Repellents and Attractants

Many stimuli attract mosquitoes, including movement, body heat, CO_2, and skin volatiles. Recently, targeted mutations in an obligate coreceptor of the odorant-binding protein receptors, orco, led to the generation of orco knockouts (48). These mosquitoes lost their host-seeking preference for humans and were refractory to the effects of DEET (*N,N*-diethyl-*meta*-toluamide). Importantly, high-throughput screening identified orco agonists that interfere with its function and that may interfere with host seeking thousands of times more effectively than DEET (93, 94). Another high-throughput screen discovered both agonists and antagonists of cpA, a mosquito neuron that is critical for detection of human skin odor (53). Antagonists blocked mosquito attraction to human stimuli. Agonists

increased attraction, suggesting that they could be used as bait to trap and control local mosquito populations. Field studies of the above chemicals in addition to structural analysis of orco and other olfactory proteins from vector mosquitoes will help in the design of more effective, targeted repellents that are less toxic to the human population and are environmentally friendly.

The identification of powerful attractants and the development of effective traps also provide opportunities for control of vector-borne disease. Mosquito traps have been developed that utilize CO_2, octenol, and human skin volatiles to attract and remove disease vectors from the environment. Detailed molecular and structural analysis of odorant-binding proteins and their preferred ligands will provide important information for the design of powerful attractants. High-throughput assays will need to be developed using recombinant odorant-binding proteins and insect cell culture to probe interactions between vector sensory proteins and candidate attractant molecules.

Wolbachia—The use of biological control as a method to reduce mosquito numbers as well as to reduce the transmission of arboviruses by mosquito vectors is a relatively novel and creative intervention technique. Mosquitoes infected with the obligate intracellular bacteria *Wolbachia* are unable to successfully reproduce due to the phenomenon known as cytoplasmic incompatibility. *Wolbachia* is naturally present in several mosquito species, including *Aedes albopictus* and *Culex pipiens*, though it is not present in *Aedes aegypti*. The release of *Wolbachia-infected* males can reduce *Culex* spp. mosquito populations in nature, and new models predict that population replacement strategies could successfully establish dominant *Wolbachia*-infected mosquitoes (95, 96).

Naturally occurring strains of *Wolbachia* can also restrict salivary gland infection of *Aedes albopictus* with DENV and limit transmission, because the number of infectious particles is greatly reduced in the saliva of mosquitoes infected with *Wolbachia* (97). In addition, *Culex quinquefasciatus* mosquitoes are less susceptible to WNV when they are infected with *Wolbachia* and are less able to transmit the virus (98). Anon-native *Wolbachia* infection of *Aedes albopictus* has also been shown to inhibit CHIKV infection (99). Although *Aedes aegypti* is not naturally infected with *Wolbachia*, several groups have successfully generated mosquitoes with stable, inheritable infections (100, 101) and have shown that these infected mosquitoes are resistant to DENV and CHIKV infection (102–104). Field trials have already been initiated to test whether *Wolbachia*-infected *Aedes aegypti* will be less prone to DENV infection in nature; these trials are based on the release and establishment of infected mosquitoes in Australian populations and follow-up to assess whether the endosymbiont remains in the local mosquito population over time. Trials with *Wolbachia* suggest that biological control of arboviral disease may play a key role in disease mitigation (102, 105). Time will tell whether the *Wolbachia* technique will continue to suppress arboviral pathogens. It is possible that the targeted viruses will evolve resistance or that the effect of *Wolbachia* on the vector will wane.

Other Viruses

Attempts have also been made to utilize arboviruses as a biological control measure, either to inhibit the replication of pathogenic arboviruses or to limit the life span of vector mosquitoes (106, 107). A genetically altered mosquito virus could also be used as a means to deliver a lethal or inhibitory gene (108). The focus has been on densoviruses, but a rapid increase in the discovery of insect-only flaviviruses may lead to the identification of viruses that effectively compete with the DENV life cycle yet do not cause disease in humans (42, 106). Use of viruses as a biological control agent may benefit from their inherent high mutation rate, which may compete with adaptation of pathogenic arboviruses, and their ability to rapidly spread through a local mosquito population. Mutations may also be selected or engineered that facilitate effective competition with pathogenic arboviruses. However, recombination events between insect-only and pathogenic arboviruses must be taken into account, which could theoretically lead to the development of novel human pathogens. Comprehensive studies should first be performed to elucidate the genetic changes that are required for insect-only arboviruses to adapt to a mammalian host.

Transgenic Mosquitoes

Another biological intervention to control mosquito populations and reduce their capacity for arbovirus infection is genetic modification. One method, called release of insects with dominant lethality (RIDL), has successfully created insects that carry a dominant-lethal gene; this technique involves males delivering female-acting transgenes to the mosquito population. Transgenes currently in use include those to reduce flight (109) and to induce mortality with age (110, 111), and the RIDL method is now being implemented in Brazil and Malaysia (112). Another use of genetic control is to create mosquitoes with enhanced viral resistance. An example is the recent creation of mosquitoes expressing RNAi against DENV RNA, which reduced infection in expressing mosquitoes (113). Finally, transgenic mosquitoes containing bacterial homing endonuclease gene (HEG) elements have been created by using simulation modeling; these have lowered vector competence and have been predicted to eliminate mosquito populations (114, 115).

Transmission-Blocking Therapeutics

Arthropod saliva can modulate the infectivity of a number of arboviruses in vitro and in vivo (60, 63). Currently, little is known about the molecular components in mosquito saliva that are responsible for infectivity enhancement, and it is unclear whether this phenomenon occurs in the human host. Our data suggest that a saliva serine protease is responsible for enhancing dissemination of DENV to draining lymph nodes in a mouse model of infection. A serine protease inhibitor was able to completely block saliva-mediated enhanced dissemination, suggesting that prophylactic intervention with a chemical inhibitor or vaccine could significantly reduce the probability that DENV would disseminate beyond the inoculation site (63). Further research is required to conclusively identify the molecular components in vector saliva responsible for infectivity enhancement before vector saliva-based TBVs can be developed.

Importantly, no saliva-mediated transmission enhancement experiments have been performed using human subjects or tissues. Therefore, it is unknown whether saliva-

mediated infectivity or transmission enhancement is relevant to human disease. These experiments would be difficult to perform considering the danger of human infection with most arboviruses and would be further complicated by preexisting memory responses against multiple arthropod saliva proteins. Further, clinical trials testing the efficacy of saliva proteins as TBVs should take into account experimental data suggesting that immunization with certain saliva proteins can enhance virus transmission and disease (63, 78). Establishment of human explant models of saliva-mediated infectivity enhancement will be critical for understanding how mosquito saliva proteins alter the epithelium and induce migration of target immune cells, and for developing novel therapeutics and vaccines to block transmission enhancement.

Acquisition-Blocking Therapeutics

Multiple components are likely required for successful acquisition of an arboviral infection in the vector midgut. As an example, recent research identified both a soluble C-type lectin coreceptor and a human CD45 homolog receptor that facilitate WNV acquisition into midgut cells (116). It is theoretically possible to develop chemical inhibitors or vaccines that would prevent the acquisition of arboviruses by uninfected vector populations. Although this strategy would not help the infected host, it would reduce the number of infected vectors within a population, mitigating the total number of human infections. This would be of huge importance from a public health perspective. Future research is required to identify target molecules that are required for acquisition of arboviruses in the mosquito midgut, and to develop chemical or biological interventions.

CONCLUSIONS

The impact of arboviral diseases is global in scope and has become a great concern due to the expanding geographical range of many mosquito species. Many arboviral diseases occur during unpredictable epidemics that increase the difficulty of controlling these infections. Further, after decades of research, a traditional vaccine is not available for DENV, which is arguably the most important arbovirus causing disease in the world today. Novel targets are desperately needed to combat arboviral diseases. In recent years, there has been a shift in the fight against arboviral disease toward a focus on vector-based instead of host- or virus-based therapies. These therapies span a wide range of technologies, some of which are being tested in the field and some of which are still in their infancy in the molecular biology lab. In the near future, we can expect to see a new arsenal of technologies available to combat arboviral disease, including transgenics, biological control agents, second-generation repellents, and transmission- and acquisition-blocking vaccines, among other possibilities.

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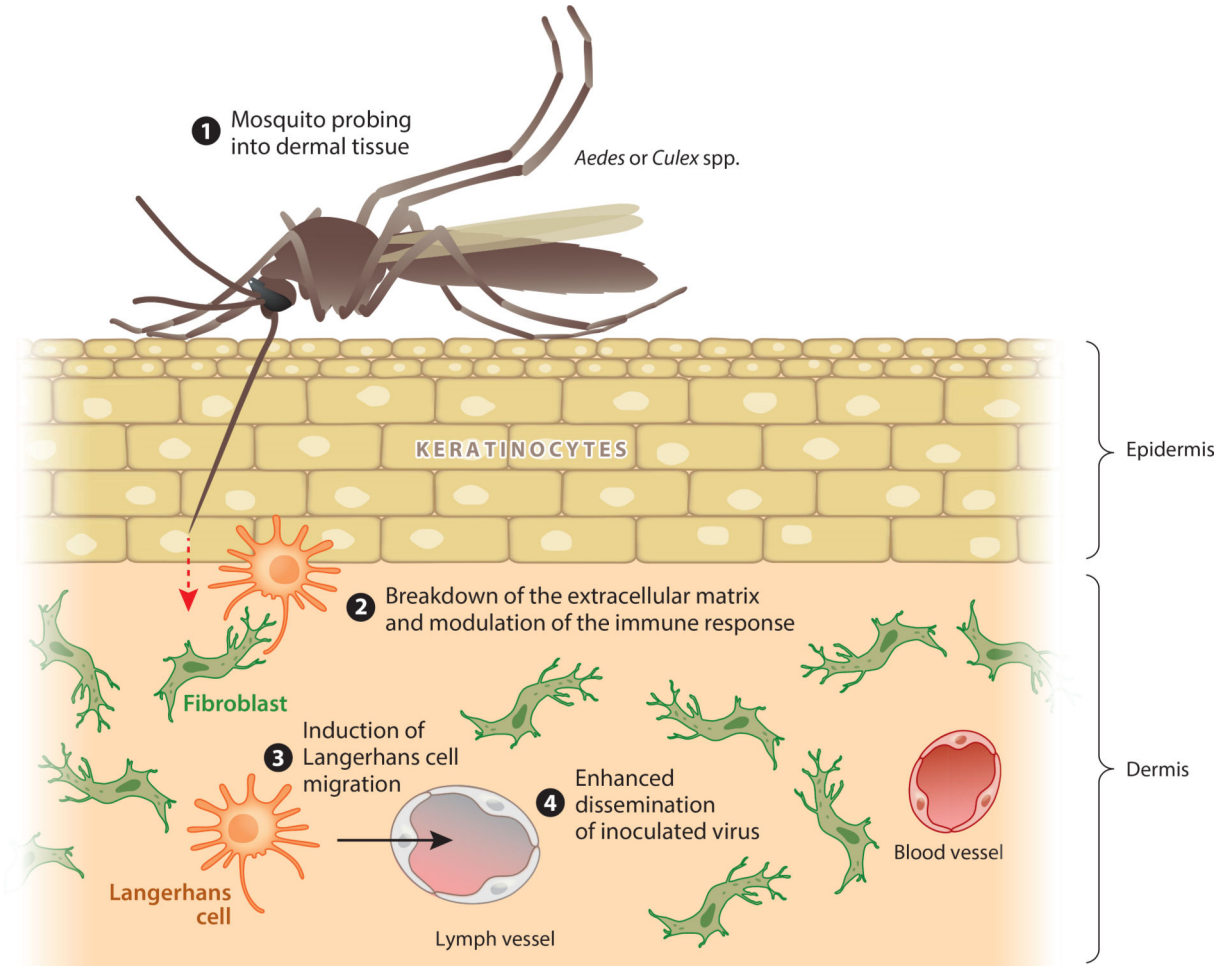

Figure 1.

Model of saliva-mediated infectivity enhancement. ❶ Infected mosquitoes inoculate virus-laden saliva mostly into host dermal tissue during probing for a blood meal. ❷ Saliva serine proteases break down dermal extracellular matrix, which modulates the immune response. ❸ Langerhans cell migration is induced, which increases the probability of interaction with immobilized virions. ❹ Infected Langerhans cells migrate to draining lymph nodes, thereby enhancing the dissemination of virus into the host.

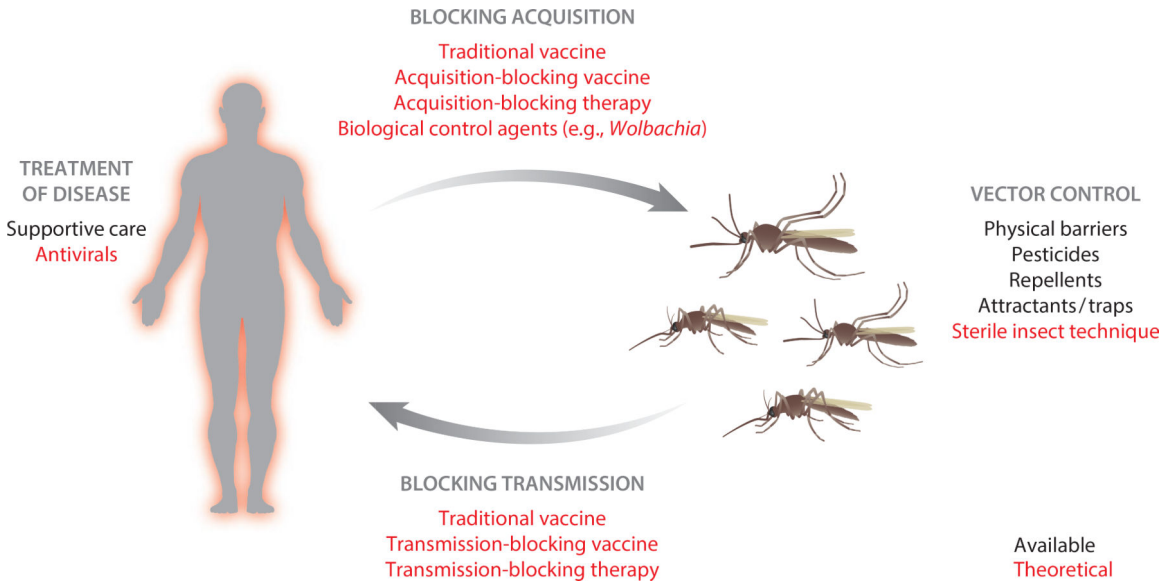

Figure 2.

Available (*black*) and theoretical (*red*) interventions to combat arboviral disease. Physical barriers, pesticides, repellants, and attractants/traps are the only methods available to prevent arboviral diseases. Supportive care is the only treatment option available in most cases. The development of a traditional vaccine for dengue virus has been problematic due to antibody-dependent enhancement. New research has paved the way for vector-based interventions including acquisition- and transmission-blocking vaccines and therapeutics, biological control agents, and the release of genetically altered, sterile mosquitoes.

Table 1

Mosquito-borne arboviruses that cause disease in humans

Arbovirus	Main vector(s)[a]	Main reservoir(s)	Endemic region(s)
Dengue virus	*Aedes*	Primates, humans	Africa, Asia, South America, Pacific
West Nile virus	*Culex*	Birds	Europe, North America, Africa, Asia
Yellow fever virus	*Aedes*	Primates, humans	Africa, South America
Japanese encephalitis virus	*Culex*	Birds, pigs	Asia
St. Louis encephalitis virus	*Culex*	Birds	Americas
Chikungunya virus	*Aedes*	Primates, bats, rodents	Africa, Asia
Venezuelan equine encephalitis virus	*Culex, Aedes*	Rodents	Americas
Ross River virus	*Culex, Aedes*	Mosquitoes	Australia, New Zealand
Eastern equine encephalitis virus	*Culex, Aedes*	Birds, rodents	Americas
Western equine encephalitis virus	*Culex*	Birds	Americas
O'nyong-nyong virus	*Anopheles*	Mosquitoes[b]	East Africa
Rift Valley fever virus	*Culex, Aedes*	Sheep, cattle	Africa, Asia
Murray Valley encephalitis virus	*Culex*	Birds	Australia, New Guinea
Orungo virus	*Anopheles, Aedes*	Mosquitoes[b]	Africa
La Crosse encephalitis virus	*Aedes*	Squirrels, chipmunks	North America
Sindbis virus	*Culex*	Birds	Europe, Africa, Asia
Vesicular stomatitis virus	Widespread[c]	Widespread[c]	Americas

[a] Only the genus of each main vector is listed.

[b] Other reservoirs may exist.

[c] More than three vectors or reservoirs have been identified.

Table 2

Effects of mosquito saliva, salivary gland extracts, and salivary gland proteins on arbovirus infectivity

Arbovirus	Vector	Model	Infection route	Infectivity	Candidate	Immunomodulation	Reference
Dengue virus	*Aedes*	C6/36 cells	In vitro	Inhibited	AAEL000598	NA	28
		Keratinocytes	In vitro	Enhanced	NA	Suppressed IFN, AMP	74
		Keratinocytes	In vitro	Enhanced	34-kDa protein	Suppressed IFN, IRF, AMP	62
		Mouse	Mosquito bite	Enhanced	NA	Long-term effects on IFN and others	69
		Mouse	Intradermal	Enhanced	NA	Suppressed TLR7, RelA, IFN, IL-10	71
		Mouse	Intradermal	Enhanced	NA	Suppressed IFN	70
Dengue virus, West Nile virus	*Aedes*	Fibroblast, mouse	In vitro, intradermal	Enhanced	AAEL005718	Suppressed TFN-α	63
West Nile virus	*Aedes*	Mouse	Intraperitoneal	Inhibited	AAEL011045	NA	26
		Mouse	Mosquito bite	Enhanced	NA	NA	60
		Mouse	Mosquito bite	Enhanced	NA	Reduced T cells, suppressed IFN	72
	Culex	Mouse	Mosquito bite	NA	D7	NA	78
		Mouse	Mosquito bite	Enhanced	NA	NA	61
Rift Valley fever virus	*Aedes*	Mouse	Intradermal, mosquito bite	Enhanced	NA	NA	64
Chikungunya virus	*Aedes*	Mouse	Mosquito bite	Enhanced	NA	Induced IL-4, suppressed TLR3	57
Cache Valley virus	*Aedes*, *Culex*	Mouse	Mosquito bite	Enhanced	NA	NA	75
La Crosse encephalitis virus	*Aedes*	Deer, chipmunk	Mosquito bite	Enhanced	NA	NA	66
Western equine encephalitis virus, St. Louis encephalitis virus	*Culex*	Bird	Mosquito bite	No change	NA	NA	67
Vesicular stomatitis virus	*Aedes*	Mouse	Mosquito bite	Enhanced	NA	Enhanced seroconversion	65
		Fibroblast	In vitro	Enhanced	NA	Reduced IFN-α	73

Arbovirus	Vector	Model	Infection route	Infectivity	Candidate	Immunomodulation	Reference
Sindbis virus	*Aedes*	Mouse	Intradermal	NA	NA	increased IL-10 and IL-4	59

Abbreviation: NA, not applicable.