THE VIABILITY OF TRANSGENESIS FOR CONTROL OF MALARIA

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Abstract

Malaria is a serious problem and a reason for severe mortality especially in developing countries. Several techniques such as insecticides, drugs, sterile insect technique, etc. have been employed by researchers to eradicate the lethal disease. However, the parasites and vectors gained significant immunity against the treatment. Incorporation of genetically modified vectors (GMVs) into disease control could be promising. Genetic modification (GM) of mosquitoes offers opportunities for controlling malaria. Transgenic strains of mosquitoes have been developed and evaluation of these to 1) replace or suppress wild vector populations and 2) reduce transmission and deliver public health gains are an imminent prospect. Identification of promoters, effectors, and reporter genes affecting malaria in mice further developed hopes for this process. This review focuses on malaria and the use of transgenesis for its prevention and cure, along with highlighting the associated challenges. Unlike other current therapies, there are no precedents (yet) of successful transgenesis application, leading to the classification of this strategy as linear technology-push, but researchers are optimistic that these strategies could be further improved.

Keywords

Malaria, Genetically modified vectors, Technological challenges, and genetic modification
INTRODUCTION

Insect transmitted diseases have been posing serious health problems and are one of the leading causes of mortality in many developing countries (1). These emerging and resurgent vector-borne diseases can give rise to infectious diseases affecting crop plants, animals, and humans (2). Plants are shown to produce toxic or inhibitory substances as an aid against insects and from the past decades, even humans have developed various insecticides such as dichlorodiphenyltrichloroethane (DDT) (3). Genetic process such as sterile insect technique (SIT), in which radiation-sterilized males confer sterility to the pest population on release, is expanding for pest control (4). However, in a wider sense, it is not very desirable to look for preferences to purge insect population as they may occupy an imperative niche in the food chain, but it is required to develop strategies to overcome the diseases spread by insect pests (2). Incorporation of genetically modified vectors (GMVs) into disease control programs could be promising. Germ-line transformation in insects of medical importance is already feasible in Aedes, Anopheles, Culex, Rhodnius, and Glossina spp. Paratransgenesis, in which host symbiotic bacteria are genetically modified to kill the parasite, is already used to control African and American forms of typanosomiasis (sleeping sickness and Chagas disease) (5). Genetic modification (GM) of mosquitoes offers opportunities for controlling malaria. Transgenic strains of mosquitoes have been developed and evaluation of these to 1) replace or suppress wild vector populations and 2) reduce transmission and deliver public health gains are an imminent prospect (6). The initial steps for transformation of malarial mosquitoes have already led to the identification of promoters, effectors, and reporter genes affecting malaria in mice (7). In mosquitoes, the drive system is based on genetic phenomena (e.g., competitive displacement, reduced heterozygous fitness, under-dominance, and meiotic drive) and systems based on exploitation of symbiotic microorganisms, viruses, and transposons (8).

Malaria – a serious problem

Malaria is one of the major causes of death in children in the first 28 days of life (9). Nchinda (1998) reported an estimate of 300-500 million cases per year with 1.5-2.7 million deaths per year and also documented that African children below 5 years constitute 90% of the deaths (10). Until the late 1960s, malaria was found in parts of Europe, USA and north Australia along with its base in tropical areas of Africa, Latin America and Asia. After that, it has spread across sub-Saharan Africa, Central and South America, Middle East, the Indian subcontinent, South and South-east Asia, Papua New Guinea and some islands in the West Pacific. In fact, malaria has affected about 100 countries and about one-third of the world’s population is at stake (11). In a report by World Health Organization, it’s recorded that 5% of African children are likely to die by malaria before age 5. This constitutes 25% of child mortality rate in Africa and it’s been noticed that most of the deaths occurs by anaemia and cerebral malaria (12, 13). There were estimated 225 million cases of malaria worldwide in 2009 and in 2011; W.H.O reported 2.23% of deaths worldwide caused by malaria (14).

Since 1992 four principles are implemented and kept in mind to control malaria: early diagnosis and treatment, selective and sustainable preventive measures, vector control techniques, containment and prevention of epidemics, and building up of local capacity (11). Apart from healthcare damage, malaria is an economic burden and it has been documented that households in Africa spend €2–25 on treatment annually and €0.20–15 on prevention per month, and in small farm households in Kenya and Nigeria 5% and 13% of the household expenditure, respectively, is spent on malaria (12, 13).
Endemic countries bear a huge loss of percentage points of gross domestic product every year due to malaria (5, 12, 13).

Malaria- causative agent
Malaria is an infection caused by a single-celled parasite and is spread by female mosquito Anopheles sp. There are five species of the parasite: Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi (15,16). Plasmodium falciparum causes severe damage and is responsible for vast majority of deaths associated with malaria (17), while P. vivax, P. ovale and P. malariae generally causes milder disease (18). However, P. vivax is responsible for largest number of infections worldwide (19). P. knowlesi is a zoonosis and cause malaria in macaques but can also infect humans (20).

Life Cycle of malaria parasite
Malaria parasite has two hosts- primary and secondary. Female mosquitoes Anopheles sp. are primary hosts and they carry the Plasmodium sporozoites in their salivary glands (See Fig. 1) (21). After feeding on an infected human, the parasite gametocytes taken up in the blood differentiate into male and female gametes and fuse in the mid-gut of mosquito, where it produces ookinete that penetrates the gut lining and produces an oocyte in the gut wall. Upon the rupture of the oocyte, sporozoites are produced that migrates to salivary gland and ready to infect a human (22, 23).

When mosquito bites a healthy human (secondary host), several invasive sporozoites introduce in the skin. They fight against the immune system and those who survive then find a blood vessel leading to liver. It has recently been shown that the sporozoites travel by a continuous sequence of stick-and-slip motility, using the thrombospondin-related anonymous protein (TRAP) family and an actin–myosin motor (24, 25). These sporozoites migrate to hepatocytes and divide and grow within parasitophorous vacuoles to form merozoites. This is called a pre-erythrocytic phase with little or no pathological actions (See Fig. 1) (21, 26). These merozoites eventually released into blood stream and initiate blood stage of infection thereon (27). Within red
blood cells, the parasite expands rapidly with a sustained cycling of the parasite population modifying the cell membrane permeability and cytosolic composition. During each cycle, each merozoite grows and divides within a vacuole into 8-32 fresh merozoites, through the stages of ring, trophozoite, and schizont. At the end of each cycle RBC ruptures and release new merozoites that, in turn, infect more RBCs (24, 25, 26).

Current therapies and genetic approach
Three basic strategies have been attempted to contain insect borne diseases: 1) treat infected people with drugs that kill the pathogen (e.g. chloroquine for...
malaria); 2) control insect vector populations (e.g. Insecticides); and 3) develop vaccines that prevent infection (28). The only successful vaccine against arthropod-transmitted pathogen is yellow-fever vaccine and even in this case, the disease has not been eradicated. Also, the research for vaccines against malaria and dengue fever has failed considerably. Two approaches are considered: 1) population suppression and 2) population replacement (29). Population suppression is achieved through insecticides like DDT, elimination of breeding sites or interference with reproduction (28) However, insects are now becoming resistant against these line of attacks (30, 31) and even other biological methods, such as the sterile-insect techniques (SIT), have been rendered less effective by changes in insect behavior (32). This integrated approach with a combination of traditional control methods, preventive measures and pharmaceutical regimes has been utilized by vector control programs in past (33), but not that they have shown to be limited in their success against the diseases, it necessitates the inclusion of an alternative control method. As Knols and Bossin (2006) put it “The continued threat of vector-borne diseases calls for both reactive and proactive efforts to mitigate the significant morbidity and mortality they cause” (34, 35).

High genetic diversity or genetic virtuosity, variability of potential target molecules, and intracellular sequestration are strategies adapted by pathogens to evade immune attack (28). Second action is to attack malaria pathogen directly rather than killing mosquito. Since the advent of genetically modified organism (GMO), research has directed to use the opportunity to control insect borne disease by creating lines of genetically modified disease vectors (6, 29, 36). Genes that can alter the behavior and biology of insects are identified and when these genes are inserted into the insect’s genome, they are called transgenes and the insect is described as transgenic insect. Introduction of transgenes into the population of wild mosquitoes will render the mosquitoes resistant to infection by the malaria parasite (28). Gene drive is a process by which the transgenic mosquitoes must be assimilated into the wild malaria-transmitting mosquito population. Gene drive requires introgression of anti-parasite genes into the insect vector populations. Such genes, if present at high enough frequencies, will impede transmission of the target parasites and result in reduced human sickness or death (37). Hopes for this possibility is raised due to reporting of genetic variability for mosquitoes against the malaria parasite (38) i.e. there is an observation that most species of mosquitoes do not transmit malaria, and even among the species that do, many individual seem incapable of transmitting the disease and are „refractory”. Alteration in genes responsible to permit malaria transmission in mosquitoes may, then, help in rendering mosquito population refractory to the parasite, eventually halting malaria transmission (39). For instance, it is noted that melanotic encapsulation is one of the reasons for this refractoriness (40). Genetic manipulation would then be achieved through identification of genes responsible for encapsulation, melanization and death of early malarial infection in mosquito (41, 42) and identification of possible mechanisms of introducing these genes into mosquito genome is critical (43, 44).
**Transgenic insects**

*Drosophila melanogaster* was the first multicellular organism to be stably transformed (45). The same germ-line transformation principle is used currently to work in insects (46). In brief, the embryo is injected with two DNA-constructs: 1) Containing gene of interest and gene encoding selective marker, both driven by separate promoter and both sequences are flanked by inverted repeats of a transposable element, and 2) Containing transposase enzyme that recognizes inverted repeats and catalyses the insertion of the intervening sequences into insect’s genome (28). The final result is the integrated host genome with the gene of interest and marker gene (…allows identification of transformed individuals e.g. eye colour, GFP). The integrated DNA is stable and transmitted in a mendelian manner from one generation to another. However, population replacement or reduction should occur in short time (8) and therefore, it should be linked to mechanisms for selection (e.g. meiotic drive) or the traits itself providing a fitness advantage (29).

Promoter involved in expression drive should be strong for abundant transcription. Out of the two types: 1) Ubiquitous, and 2) Tissue-specific promoters, the latter is observed to be advantageous as it is restricted to specific tissue (28). Strong tissue-specific promoters that have been characterized in mosquitoes include gut carboxypeptidase (47), fat body vitellogenin (48) and gut peritrophic matrix protein 1 (PM1) (28). Similarly, effector genes are equally important. They could be: 1) Gene products interfere with the development of pathogen. For e.g., SM1 (a peptide occupying salivary-gland and midgut receptors for the malaria parasite) (49), Phospholipase A2 (PLA2; protein that interfere with malaria ookinete invasion of the midgut (50), 2) Gene products interact with pathogen. For e.g., gene encoding monoclonal antibodies that block parasite’s development by binding to its outer surface (51), 3) Gene products kill the pathogen. Examples are peptides from the host immune system such as defensins and cecropins and peptides from other sources such as magainins, Shiva-1, Shiva-3 and gomesin (52). Another possible strategy is to reduce vector competence by manipulation of its immune genes, for instance by using RNA interference or „smart sprays“ (28).

Development of effectiveness in the aforementioned technology (transgenesis, promoter characterization and effector-gene identification) permits creation of genetically modified mosquitoes impaired to transmit parasites i.e. refractory mosquitoes. A variety of engineering refractory mosquitoes are currently studied for malarial (53). An early example is *Anopheles Aegypti* that was developed to express defensin in the haemolymph (48). Another creation was a single chain monoclonal antibody reported to recognize sporozoite surface protein inhibiting invasion of the salivary gland (54). Transformation by gene encoding SM1 and PLA2 inhibiting parasite development and showing reduction in vectorial competence is also reported in transgenic mosquitoes (55, 56). Ito and colleagues showed that transgenic mosquitoes were less susceptible to infection (oocyte load) and had fewer sporozoites in their salivary glands. They suggested that where mosquitoes carry
less than five oocytes, inhibition of transmission is very effective (55). Kim et al, 2004 reported 60 % reduction in transmission of malaria parasite in transgenic *Anopheles gambiae* expressing cecropin from a carboxypeptidase promoter (52). Another method for generating refractoriness involves discovering genes that govern refractoriness in natural population (7). From the above discussion, it is evident that mosquitoes can be genetically modified to reduce vector competence, however, it requires overcoming certain hurdles to provide desirable efficacy.

**CHALLENGES**

**Parasite resistance Fitness and Gene drive**

Parasites have heterogeneous genome and favors selection of individuals to overcome barrier such as drugs and effector gene products. It is therefore, important to insert more than one effector gene to block parasite development by different mechanism (28).

For effective transformation in mosquitoes, it is important that there is least possible detrimental effect on their survival and reproduction (fitness load) (28). However, till now, its not yet achieved. Mosquitoes with PLA2 gene-transformation show reduced egg production (47, 55, 56). Mosquitoes with GFP from actin promoter may also have fitness disadvantage (28). Introduction of novel phenotypic traits in transgenic mosquitoes may induce these fitness impairments impeding gene drive and spreading of transgenes (57). Expression of effector genes, especially using ubiquitous promoters, may be detrimental for the mosquito. Also, transgenes may interfere with gene expression of mosquito strain because of random genomic insertion. This intentional mutagenesis may be naturally selected and can be overcome by selection on lines with higher fitness. Whatever approach is adopted in the coming years, compensation for fitness loss, preferably by conferring fitness advantages to transgenic lines is desirable (28).

Effective transformation also requires efficient gene drive system, which is undoubtedly, a major technical issue. Many features are required to make a gene drive ideal for competitive displacement of population (28, 29). For instance, gene drive should compensate any fitness load incurred by transgenesis (6, 9, 28, 55); it should carry large fragments of DNA to accommodate all the elements controlling driver characteristics; it should have minimal side-effects and incur elimination of any unforeseen phenotype and ecological effects (29). Transposable elements are widely appreciated for gene drive as they not only rapidly move and propagate within the genome, but also colonize other genome through horizontal transfer (29). To date three malaria vectors have been transformed using transposable elements: the Asian vector *Anopheles stephensi* (44), the Latin American vector *A. albimanus* and African vector *A. gambiae* (55, 57, 58). Another mechanism in insects is meiotic drive, where genes (Drivers) can overrule random segregation of chromatides and forcefully drive their inheritance through populations (59). So, if the trait is linked to one of these aggressive genes, it can rapidly and effectively be spread among the mosquito population. Meiotic drive distorting sexual behaviour is detected in mosquitoes raising hopes for its use in GMM strategies (60). However, the molecular mechanism of meiotic drive is complex involving multiple genes and is still elusive. Intracellular symbiont such as Wolbachia is also considered promising for gene drive using cytoplasmic incompatibility (CI) (61). Although, it has not been identified in mosquitoes yet, research shows experimental transfer of bacterium over genus barriers including complete CI in the new host (62).

Based on these, Jacobs-Lorena (2006) formulated priorities for research in transgenesis of insects (28)-

“...a method to drive effector genes into field mosquito populations needs to be devised.

- The efficiency of *An. gambiae* transformation needs to be improved.

- Anopheline mosquitoes cannot be stored frozen or desiccated. Establishment of repository centres for transgenic lines is desirable.

- Whenever possible, work should be conducted with the organisms that are most relevant to human disease (*An. gambiae, P. falciparum)*.”

**Translational challenges**

There are several translational challenges surrounding transgenesis for malaria control (See Fig. 2) (6). Ethical, legal and social issues involved in transgenesis are noteworthy (63).

Even though transgenesis is promising in laboratory and research bench to eradicate malaria, it is essential that countries evaluate and adopt this approach as a potential tool for malaria control. This will increase the investment in knowledge and skills acquisition with respect to transgenic mosquitoes. Ultimately, it aims to transfer the problem-ownership to the disease endemic country (DEC) (64). Another thing to consider is containment and risk management. Although, guidelines are available for handling, transport, and laboratory confinement for transgenic mosquitoes, no such guidelines are drafted for DEC (6).

In the above sections, several challenges that transgenic mosquitoes will face in future are discussed. Although, forecasting is often inaccurate, some features that may affect GM mosquitoes are emphasized by Knol et al, 2007 (6): 1) By 2025, 90 % of world’s population will live in developing countries, 2) 52 % of African population will live in urban environment, 3) Environmental modification may aid in malaria control, 4) High population density may increase the interest towards case management, personal protection measures, or larval control, 4) Genetic strategies may become attractive.
for population replacement in future and may eliminate malaria. Holistic, tedious, resource-intensive, time-consuming approach is needed to drive genetic control trials to maximize the efficiency and minimise the obstructions (6), but they will ultimately decide whether transgenesis is viable for controlling insect-borne diseases.

Figure 2 Views from the malaria research and control community (N = 196) in response to statements presented during a plenary debate at the 4th MIM conference held in Yaoundé, Cameroon, in November 2005. The debate was titled: “Is the transgenic mosquito as a weapon against malaria ever going to fly?” Black, yes; dark gray, no; light gray, no opinion; white, no answer (Adapted from 6)

Conclusion

The concept of transgenesis for malaria control is attractive. Effector genes driven through wild population via the release of transgenic mosquitoes could achieve innocuous vectors. These recent achievements shows that this is no longer a hypothesis, but a realistic research avenue. Although, many technical issues are unsolved, transgenesis is promising References

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