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Dengue: translating scientific progress into workable solutions

'Although scientists from developing countries have long recognized the public health need for research on dengue, it has too often been neglected by scientists from the developed world.'

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An infusion of funding, talent and new scientific techniques in virology and immunology has recently yielded major gains in the field of dengue research, with promising developments in the basic understanding of the biology of the virus, its mosquito vector and the pathogenesis of disease, as well as in the identification of candidate vaccines and therapeutics. Significant hurdles remain, however, in applying that knowledge to benefit the increasing number of individuals threatened by this re-emerging infectious disease. Based on a perspective of 20 years of research on dengue, the authors propose several research areas of emphasis to facilitate the translation of basic research into real world solutions.

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Key features & challenges

Dengue is an acute febrile illness caused not by a single virus, but by four antigenically related flaviviruses usually referred to as serotypes of dengue virus. As with all flaviviruses, these are small enveloped viruses containing a nonsegmented RNA genome approximately 10 kb in length of positive (message) polarity. Dengue virus infection is acquired through a transmission cycle between invertebrate and vertebrate hosts. The invertebrate hosts for dengue virus are mosquitoes of the genus *Aedes* (*Stegomyia*), principally *Aedes aegypti*, a highly anthrophilic tropical mosquito. The vertebrate hosts are almost exclusively humans; nonhuman primates are infected in nature, but play little, if any role in the distribution of human infection [1].

In humans, there is a wide spectrum of clinical manifestations of dengue infection. The most common clinical presentation is an uncomplicated and self-limited febrile illness (dengue fever [DF]). A plasma leakage syndrome accompanied by hemorrhagic diathesis (dengue hemorrhagic fever [DHF]), is observed in a small percentage of cases. DHF may lead to shock and death. Although DHF is infrequent, owing to its severity and the large population at risk, DHF (whether present or suspected) plays a large role in the public health problem of dengue.

While many factors contribute to an individual's risk for DHF during dengue infection, one of the most significant observations was the recognition of the importance of prior immunity. This becomes important in the case of dengue, since infection with one dengue virus serotype provides only transient (several months) protection against the other serotypes. Therefore, sequential infection with multiple different dengue virus serotypes is possible and, due to the increasing global transmission of all four dengue virus serotypes, this occurs frequently among the population in heavily affected tropical developing countries [2]. Although the 'sequential infection hypothesis' of DHF was initially controversial, it has been established in multiple

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epidemiologic settings that DHF risk is markedly higher in secondary dengue infections than in primary dengue infections. This association is therefore a foundation for understanding the pathogenesis of DHF and for designing strategies to prevent and/or treat dengue infection.

A neglected research area

Although scientists from developing countries have long recognized the public health need for research on dengue, it has too often been neglected by scientists from the developed world. This was not always the case. Many of the early advances in our understanding of dengue were made by scientists from developed countries, especially the USA and Japan, including the demonstration that dengue was caused by a filterable agent, and the recognition of *A. aegypti* mosquitoes as the vector. Much of that research was conducted by investigators based in the US military; the significant impact of dengue-related morbidity on troop readiness during deployments in Asia and the Pacific (and seen more recently in the Middle East and Haiti) provided a strong incentive for clinical and basic research towards the development of a vaccine. Nevertheless, as recently as the 1990s, the field was represented at international scientific meetings by a small (but close) group of researchers distributed widely across the globe, and few US scientists had National Institutes of Health (NIH) funding for dengue research.

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Recent developments

Research on dengue has recently benefited greatly from a concurrence of expansions in funding, interest and technology. NIH support for research on viral hemorrhagic fevers has increased with the biodefense initiatives, and research on dengue has been included in these efforts. In addition, a Pediatric Dengue Vaccine Initiative [101] was recently established with support from the Gates Foundation, and has supported basic and applied research on dengue. The governments of other countries including Singapore, Taiwan, Cuba and Thailand, and corporations such as Novartis and Aventis, have also made major commitments to funding for basic and applied research on dengue.

The increased availability of funds has come at a welcome time for the field. First, it occurs at a time when US Department of Defense funding for basic research has experienced cutbacks. In addition, the number of trained molecular virologists has continued to increase, while some alternative areas of research have been constrained by security considerations (e.g., Japanese encephalitis and Venezuelan equine encephalitis viruses) or other factors (e.g., pending controls on stocks of wild-type polioviruses).

These changes have already paid substantial research dividends. Understanding of dengue viral biology has increased greatly with the solution of the structures of a number of critical viral proteins. The structures of the envelope glycoprotein have been reported both for the dimer found on the mature virion, and for the trimer formed during the process of fusion of viral and host endosomal membranes [3]. This information has led to the identification of candidate inhibitors of viral binding and/or entry. Protein structures are now also known for the viral protease, helicase and methyltransferase domains [4,5]. Each of these enzymes is essential for viral replication, and it is anticipated that high-throughput drug screening approaches will allow the identification of candidate inhibitors of these steps, as has already been accomplished for HIV and hepatitis C virus.

A second area of recent research accomplishments is viral–host cell interactions. Global effects of viral infection on host cell gene expression have been defined using differential display and microarray technologies [6]. Also, more specific effects of viral proteins on gene expression and function have been characterized. These approaches have identified host cell immune responses, as well as viral adaptations to overcome these defenses, particularly the Type I interferon response [7,8]. Using techniques for manipulation of the dengue viral genome developed over more than 10 years, it is hoped that these viral counter responses can be mutated to produce better live attenuated viral strains for use in future vaccines.

Clinical research on dengue has seen significant accomplishments. Researchers in several regions have followed ambitious study protocols and applied complex molecular and immunologic techniques to address the dynamics of transmission, pathophysiology of disease and treatment approaches [9–12]. These studies have helped to identify best practice methods for the study of hospital-based ill subjects, population cohorts and epidemiologic clusters. Studies of acutely ill subjects have improved our understanding of the kinetics of viral replication and immune responses *in vivo*. They have demonstrated that severe disease is associated with high viral loads early in illness, as well as the progressive induction of a vigorous cascade of T-lymphocyte activation and cytokine production [9]. Prospective cohort studies have revealed complex patterns of cocirculation of different viruses within regions, and have begun to identify patterns of antibody and T-lymphocyte responses that affect disease severity during subsequent dengue infections [11,13,14].

Lastly, progress has been made towards the development of a vaccine against dengue [15]. Most advanced among the vaccine approaches is the development of a tetravalent vaccine based on live attenuated virus strains derived from a traditional tissue culture approach. Early phase clinical studies have yielded encouraging data on safety and immunogenicity, and a candidate vaccine may enter Phase III field efficacy studies within the next few years. Development of live attenuated viruses through reverse genetics based on either a mutated dengue virus genome or the yellow fever virus vaccine strain has been successful in early phase clinical studies, and may prove to be an alternative strategy for a tetravalent vaccine. A variety of other academic and industry groups are pursuing vaccine development based on recombinant proteins and/or DNA vaccination, but these approaches face many more short-term hurdles.

Research needs

Considerable work remains to be performed before these research accomplishments can produce tangible benefits to public health in countries most affected. The current status of research on dengue points to a scenario in the near future where several candidate therapies or vaccines will have been proposed based on elegant *in vitro* studies, but they will be unable to advance into pivotal clinical studies due to scant preclinical data, uncertainties as to the appropriate design for relevant clinical trials or lingering concerns about the potential to exacerbate disease. Therefore, it will be crucial to maintain an adequate level of funding to address several key research areas.

The lack of a suitable experimental animal model for dengue disease continues to be a major obstacle to development of vaccines and therapeutics against dengue. Many efforts have been made to study dengue infection in laboratory mice, including recent work in strains deficient in antiviral responses, and also in nonhuman primates. While interesting results have been reported, the models described to date continue to be deficient in key respects, particularly, the failure to reproduce the plasma leakage syndrome of DHF, the association of DHF with secondary dengue virus infections and the relationship of disease with immunologic responses. Studies of animal models with intact immunologic responses, and of responses to sequential dengue infections, should be a priority. Research in this area should optimally include outcome measures that are relevant to dengue disease in humans.

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Continued emphasis on clinical studies of dengue disease pathogenesis should also remain a priority in order to address new and unresolved questions, inform efforts to develop new animal models and maximize the safety and scientific value of clinical trials of candidate vaccines and therapeutics. A major issue for candidate antiviral agents for the treatment of dengue is whether inhibition of viral replication will affect disease outcome. Although the association of higher viral burden with more severe disease would suggest that therapy would be of value, the kinetics of viremia may make targeted antiviral therapy impractical. Viremia titers decline rapidly for several days before plasma leakage occurs in DHF. Therefore, there is a need for more detailed analysis of *in vivo* viral replication to identify time points where antiviral agents could be of use. The tissue (and cell type) distribution of dengue virus during infection has been defined only to a very limited extent to date; comparison of primary and secondary infections of varying disease severity is sorely needed. Dengue infections in infants have also not been well characterized. The occurrence of DHF during primary dengue infections in this population has been taken as particular evidence of the importance of antibody-dependent enhancement of infection, and this epidemiologic setting may be ideal to address the effect of pre-existing antibodies on viral replication.

Recent studies have also provided additional evidence that differences in genetic characteristics of the population and of the predominant circulating viral serotypes and strains have a significant effect on the severity of disease observed. A better understanding of viral and host genetic influences on disease could help to define new targets for therapy or strategies for engineered attenuation of virus strains. Therefore, studies of disease pathogenesis in different parts of the world, and extending over multiple outbreak periods, should be supported. These studies should follow the best practices of recently published studies, including appropriate designs to maximize the detection of the infecting virus strains, in order to optimize the potential to compare results across studies.

Further investigation of the immune response to natural primary and secondary dengue infections, as well as to candidate vaccines, must also remain a priority, because the evidence that immune responses contribute to the outcome of infection is so compelling. Identifying immunologic correlates of protective immunity or, conversely, of pathologic immunity (i.e., increased risk for severe disease) is difficult in populations in endemic areas, but this will be critical to the evaluation of field studies of candidate vaccines. Studies to date have provided only preliminary clues to these associations, but have also shown the low predictive value of assays such as the standard plaque neutralization assay. There is a need for the development of novel assays of antibody specificity and/or avidity and improved assays of cell-mediated immune responses that are more easily standardized, and for the validation of these assays as markers for protective and/or pathologic immunity. These studies will need to extend for years after initiation of field vaccine efficacy studies, because any potential risks may be time dependent.

Although the association of DHF with secondary dengue virus infection is a potential risk for a vaccine, the ability of primary dengue virus infections to produce long-lasting resistance to that serotype provides a natural example of protective immunity. With this target in sight, there is reason to hope that vaccine development will be successful, if we are able to understand how to effectively reproduce this natural example.

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