Global Perspectives on Dengue Research

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Abstract

Dengue viruses infect nearly 100 million human beings each year living in 110 countries spread over all tropical areas on earth. Tens of millions of dengue illnesses occur annually including the hundreds of thousands of children who are hospitalized for dengue haemorrhagic fever. A health problem of this scope should be regarded as high priority and should have attracted ample funding from donors and national authorities. But such is not the case. A brief historical review reveals that there was a greater number of laboratories and a greater allocation of resources to dengue research 30-50 years ago than there is today. WHO needs to provide leadership in promoting dengue research. Each and every dengue-endemic country should realize that a sustained research capability is crucial to solve the long-term problem of dengue control. This paper provides a brief review of the history of dengue research. For several disciplines, key scientific questions are listed. Answers to these research questions are urgently needed to cope with the dengue problem.

Key words: DF/DHF, global perspective, research, control

Global status of dengue and dengue haemorrhagic fever

Dengue viruses circulate in nature as four antigenically-related serotypes, the only such group among the arthropod-borne viruses. Each of the four serotypes have evolved into multiple genotypes. The viruses are maintained in nature in two cycles, a jungle cycle (presumably older) in which several sylvatic mosquito species transmit viruses to several species of sub-human primates, and an urban cycle in which the virus is transmitted predominantly by Aedes aegypti to human beings. The dengue viruses are unique in that a single dengue infection may “sensitize” individuals to severe and fatal disease accompanying infection with a second serotype.

In response to a number of 20th century phenomena, the distribution and population
of Aedes aegypti and the global burden of dengue have grown dramatically in recent decades. With burgeoning human populations, urbanization and the development of rapid transport systems, dengue fever and dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) now occur in over 100 countries and territories. They cause an estimated 50-100 million infections among the more than 2.5 billion people at risk in urban, peri-urban and rural areas of the tropics and subtropics. While the full burden of dengue infections is not known, each year it is estimated that some tens of millions of persons experience classical dengue fever and another 500,000, mainly children, are hospitalized for DHF/DSS. Death rates are as high as 5% in some areas. Dengue is now endemic in the American, Western Pacific and South-East Asian regions of WHO while some parts of the African and Eastern Mediterranean regions are affected. Prior to 1970 only nine countries in the world had experienced DHF/DSS epidemics; by 1995, the number had increased more than four-fold. In the 1950s, an average of 908 DHF/DSS cases were reported each year. For the period 1990-1998, this average had increased to 514,139 cases. In 1998, a total of 1.2 million cases of dengue and DHF were reported to WHO, including 3442 deaths.

Global status of dengue research and control

History: Dengue research began early in the 20th century before the virus was isolated. The clinical and laboratory features of dengue, the viral status of the agent, the susceptibility of monkeys and the vector status of Aedes aegypti were established in a series of well-designed human volunteer studies. Dengue viruses, types 1 and 2, were isolated in suckling mice and characterized during World War II.

Research milestones: In the late 1950s, the clinical syndrome, DHF/DSS, was described and attributed to dengue infection. A decade later, it was recognized that DHF/DSS accompanied second dengue infections and, unique in human medicine, during initial dengue infections in infants who were born to dengue-immune mothers. Subsequent epidemiological studies, a monkey model and numerous in vitro observations provided an explanatory mechanism, antibody-dependent enhancement (ADE) of dengue infection. This is based upon evidence that dengue viruses replicate in cells of mononuclear lineage in human beings. These Fc-receptor-bearing cells are efficiently infected following attachment of complexes of dengue virus and non-neutralizing IgG antibodies. From the 1970s, with the emergence of modern immunology, the role of cellular and humoral immunity, the molecular mechanisms of inflammation and the control of dengue infections were studied. During and subsequent to the 1980s, full-length sequences of the dengue genome have been described for multiple strains of all dengue serotypes. Recently, molecular genetic research has yielded engineered vaccines and rapid, highly sensitive methods to detect and study viral infections.

Facilities: During the 1950s dengue studies were performed in field laboratories maintained by the Rockefeller Foundation in Trinidad, Brazil, Africa and India, and by
colonial research institutes located in South and south-east Asia. A decade later many of these research networks were phased out and replaced by national public health laboratories, a network of U.S. military infectious diseases research laboratories (Thailand, Malaysia, Philippines, Indonesia, Peru, Brazil) and for a brief time, WHO vector research units in Thailand, Indonesia and India. Today, dengue research is supported by intramural funds in government research institutes of the larger developed countries. The biggest cadre of scientists is in the U.S. public health service (CDC and NIH) and military research laboratories. As compared with chronic diseases, dengue is a low-priority health problem for developed countries. Comparatively modest support for dengue research is provided on a competitive basis to a small number of university scientists in developed and some of the relatively affluent developing countries.

Control Programmes: During the 1940s, a historically unique mosquito control programme was initiated. By 1960, under the leadership of the Pan American Health Organization, Aedes aegypti had been eradicated from most major South and Central American countries. After this achievement, however, many of these programmes were dismantled and within two decades, the vector regained its former range.

Research questions and WHO’s role
A comprehensive research programme on all aspects of dengue and its control will entail a vast, multidisciplinary effort. In the view of the author, some of the key research questions and the role for WHO in answering them include:

Basic virology: Progress in basic virology is moving very quickly. The flavivirus field is diverse, small and dynamic. Compared with other genera, disease-producing flaviviruses are little used in the study of basic virology.

Questions: What is the function of the proteins translated from the dengue genome? Do functions differ by serotype? Genotype? What amino acids, proteins and three dimensional conformations participate in the cell entry process? What are the mechanisms of entry? Do these differ by serotype? Genotype? How do dengue viruses replicate, assemble and release from relevant human target cells?

WHO role: Use convening function of WHO to focus research on important disease or vaccine-related questions.

Viral evolution: Questions: What is the global extent of contemporary zoonotic cycles of dengue viruses? Did dengue evolve into four distinct serotypes in geographically isolated zoonotic cycles? If so, when? When did each dengue serotype escape from subhuman primates to human beings? And, how many times? Are or were dengue viruses transmitted from humans to monkeys? Are monkey dengue viruses currently being transmitted to humans?

WHO role: Jungle dengue could threaten long-term efforts to control urban dengue. If WHO committees highlight the importance of this question, it may stimulate
interest and promote and coordinate efforts to recover dengue viruses from zoonotic cycles.

**Host-virus interactions:** Questions: What cells in human beings serve as important/essential sites of dengue virus infection (target cells) during the course of dengue fever, DHF/DSS? Do target cells differ with different serotypes, genotypes, infection sequence? What cells contribute to the signs and symptoms of classical dengue fever, DHF and aberrant dengue? What is the mechanism of entry of dengue viruses into non-Fc-Receptor-bearing target cells? Why are some dengue genotypes associated with severe secondary dengue infections and others are not? Can mice or other small laboratory animals be used to study key virus-host interactions? Can in vitro systems be used to study virus-host interactions? How does antibody promote severe infections? What are the characteristics of inapparent dengue infections? Can such infections be recognized in nature? Are blacks genetically resistant to dengue infection or disease? What human gene(s) control response to dengue infection?

**WHO role:** This is difficult research. Direct funding by WHO and use of the convening function may promote and accelerate research in this area. Discovery of the human gene that controls response to dengue could lead to new drugs or powerful new methods of dengue control.

**Pathophysiology/treatment of dengue/DHF:** Questions: What are the mechanisms (e.g., target cells affected) and effector molecules that mediate major and minor clinical phenomena observed during classical dengue fever, classical DHF and aberrant dengue? Can these mechanisms or effector molecules be identified at an early stage of dengue infection permitting specific treatment to prevent onset of severe disease? Can dengue infections be identified early enough to permit early interventions? Can the treatment of DHF/DSS be improved, simplified or made more cost-effective? Can dengue antivirals be developed?

**WHO role:** Very few well-funded, high-quality research groups in the world are studying dengue pathophysiology. WHO may be able to support the creation of new interdisciplinary groups. Capable clinical researchers may be able to work on dengue if they can have access to research-level virological support.

**Vaccine development:** Current dengue vaccines under development include: two sets of tetravalent live attenuated viruses (LAV) (Mahidol/Merieux and WRAIR), a genetically-altered LAV (NIH), a dengue-dengue chimera (CDC/Mahidol), a yellow fever-dengue chimera (Acambis), an alphavirus replicon (USAMRIID), two naked DNA vaccines (Naval Medical Research Center/USAMRIID), a formalin-inactivated tetravalent vaccine (WRAIR) and numerous sub-unit vaccines prepared by commercial, university or government laboratories. Some of these vaccines are or have been tested in human volunteers.

**Questions:** What are the immunological goals of dengue vaccination? What is the herd immunity of different dengue serotypes? How do immune responses in laboratory animals compare with those in human beings? What...
laboratory methods can be used to measure and predict solid protection of vaccinated humans? Which of the above vaccines (or others) meet dengue vaccination goals? What is the efficacy of candidate dengue vaccines? What are the safety concerns of dengue vaccines? What laboratory methods can be used to measure and predict safety of dengue vaccines?

WHO role: WHO should directly address safety issues and laboratory markers of protection. WHO should monitor dengue vaccine research and promote the phase I and phase II testing of attractive vaccine candidates, particularly in children. WHO and its children’s vaccine allies should raise the funds needed to assist in scale-up, testing, production and distribution of safe and effective dengue vaccine(s).

Control – vector bionomics: A 1992 study commissioned by the Rockefeller Foundation could find no major university in any dengue-endemic country that offered graduate programmes in Aedes aegypti bionomics or control. Many national vector control programmes do not have Ph.D. medical entomologists in leadership positions. Those with graduate degrees usually have obtained them from abroad. Thus, there is no academic base to complement government programmes or provide the research that is essential to achieve optimal local control of dengue vectors.

Questions: What is the flight range, survival, biting and breeding behaviour of Aedes aegypti in different sites? Although this mosquito is one of the best-studied, important information relevant to mosquito population survival and to sustained dengue virus transmission is lacking.

WHO role: This is a critical area for WHO capacity-building and networking. Global medical entomology personnel research and personnel resources are at all-time lows.

Control methods: Many methods are available to reduce or destroy populations of Aedes aegypti. Nonetheless, since the days of Dr Fred Soper, no-one has found the right mix of methods that matches the resources available or is compatible with today’s legal systems.

Questions: Are currently available methods simply too labour intensive and expensive? Or, is the failure of aegypti control an example of systems failure? (see below). How can basic research contribute to reducing populations or destroying vectorial capacity?

WHO role: The entire field of mosquito control needs to be energized, even re-born. Leadership and funding are essential.

Control – human behaviour: Aedes aegypti breeding sites are largely man-made; humans provide the blood that promotes the survival of both mosquito and virus. The activities that promote mosquito breeding are not usually linked to emotion-laden customs or behaviours. As with malaria prevention, ignorance, traditional practices and carelessness must be overcome.

Questions: Can key attributes of behaviour-promoting mosquito breeding be identified? Can strategies be devised and tested to motivate behaviour changes? What role can education play in changing behaviour? What are the target age and sex groups? What messages work best for each age and sex group?
WHO role: Using its convening function, WHO should attempt to breathe life into this research field. Direct support of research and pilot projects is essential.

Control - systems development:
Above all, dengue control is a test of a system. Disease surveillance and awareness of illness and cost-burden are needed to motivate and inform educational interventions and to design interventions at specific localities.

Goals: Tests need to be made of ways in which the legal system can enforce and re-enforce desired behaviour. Studies need to be made of materials and devices that can be used by householders that will provide passive prevention of mosquito breeding or killing of larvae or adults.

WHO role: If WHO doesn’t stimulate and support improvement of surveillance and control systems, who will? There is some important research to be designed and supported; largely, this is an area for the Control of Communicable Diseases Division.

Control - private/public partnerships:
With sufficient funds, it can be predicted that the private sector will be able to provide effective control of Aedes aegypti.

Goals: Devise one or more pilot-scale projects to test the ability of the private sector to control Aedes aegypti to an established level. Devise programmes for the public/private sector cooperation in the control of vector and nuisance mosquitoes.

WHO role: WHO leadership is essential. This is part research and part intervention. Cooperative projects supported by WHO’s Tropical Diseases Research (TDR) and Communicable Disease Control Programmes are needed.