Development of Dengue Vaccine

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Abstract

The dengue viruses are estimated to cause several hundred thousand cases of dengue fever, dengue haemorrhagic fever and dengue shock syndrome annually. Attempts to prevent the infection focus on the development of a vaccine that would protect against all four serotypes of the dengue virus. Various biotechnological approaches are being explored, including the use of live attenuated or inactivated viruses, infectious clone-derived vaccines, immunogens vectored by various recombinant systems, subunit immunogens and nucleic acid vaccine. Three candidate vaccines are undergoing clinical evaluation and several are at the stage of pre-clinical evaluation. A WHO steering committee is conducting activities aimed at accelerating the development of vaccines against dengue and Japanese encephalitis.

Key words: Dengue vaccine, clinical evaluation, preclinical stage

Introduction

Dengue viruses are the most widespread arthropod-borne viruses. They are members of the flaviviridae family, which includes more than 70 related but distinct viruses. Among these are important aetiological agents such as those of yellow fever (YF), Japanese encephalitis (JE), West Nile encephalitis and tick-borne encephalitis. Dengue is one of the most important tropical infectious diseases. It is estimated that there are some 100 million cases of dengue fever, 500 000 cases of dengue haemorrhagic fever (DHF) and 25 000 deaths attributable to dengue annually(1). In recent decades the transmission of dengue viruses has intensified in many countries and the disease has extended its geographical range to previously unaffected areas of the South-East Asia Region, the Western Pacific Region and the Region of the Americas of the World Health Organization. In the past, the African Region and the Eastern Mediterranean Region were considered to have low incidences of dengue, but there was an upsurge of the disease in these regions.

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Development of Dengue Vaccine
during the early 1990s. Dengue has grown dramatically as a health, environmental and economic problem, now occurring in most countries. More than half the Member States of the United Nations, with a population of some 2500 million, are at risk.

Dengue viruses are classified antigenically into four serotypes. Infection with one serotype results in lifelong immunity to it but there is no cross-protection against the others. Persons living in areas of endemicity can be infected with two, three and, probably, four dengue serotypes during their lifetime. Infection with any serotype can produce clinical illness, ranging from a non-specific febrile syndrome to severe and fatal DHF/dengue shock syndrome. An immunopathological response following secondary infection of humans with a heterologous serotype of dengue virus can be a risk factor for the more severe forms of the disease. This was recently confirmed in Cuba, where an 18-year interval between a dengue virus type 1 outbreak in 1977/1978 and a dengue virus type 2 outbreak in 1997 provided an opportunity to evaluate risk factors\(^2\). All patients with severe forms of dengue, including cases of DHF and deaths, were born before the dengue virus type 1 epidemic, and nearly all experienced the secondary dengue virus infection. In contrast, almost all those who seroconverted without illness experienced the primary dengue virus infection. These observations could have implications for the development of a dengue vaccine because they suggest that a safe vaccine should be polyvalent to avoid inducing monotype-enhancing immune responses that may lead to severe manifestations of the disease.

No effective vaccine is available. Research into dengue vaccines focuses on the use of live attenuated or inactivated vaccines, infectious clone-derived vaccines, immunogens vectored by various recombinant systems, subunit immunogens, and nucleic acid vaccines.

Tetravalent live attenuated vaccine
The most advanced live attenuated tetravalent vaccine was developed in Mahidol University, Thailand, with the support of WHO’s South-East Asia Regional Office. Attenuated viruses of all four serotypes were developed by serial passage of wild-type viruses in primary dog kidney (PDK) cells or other cell types\(^3\). After intensive and stringent laboratory studies, including evaluation in animal models, the vaccine underwent clinical trials in Thailand in mono-, di-, tri- and tetravalent formats, which proved safe and immunogenic in adults and children. The vaccine proceeded to commercial development by agreement with Aventis Pasteur. A randomized, controlled, double-blind study was carried out to determine the safety and immunogenicity of batches of the vaccine produced by this company\(^4\). All formulations were safe and tolerated in humans. Vaccines immunized with tetravalent vaccine gave multivalent antibody responses, the highest antibody titres being against dengue virus type 3. A phase 1 clinical trial of Aventis Pasteur vaccine was recently completed in Thailand. After two doses, seroconversion to all four serotypes was demonstrated in most vaccinated volunteers and antiviral activity remained quite stable for at least a year.
Various reformulations of the tetravalent vaccine are being evaluated in an attempt to obtain a similar immune response to each serotype. Vaccine strains developed at Mahidol University are characterized by lower infection, dissemination rates and transmissibility in Aedes aegypti mosquitoes than those of the parent viruses\(^5\). Moreover, the phenotypes of the vaccine strains were stable and unchanged by passage in humans and mosquitoes.

Serial passages of dengue viruses in PDK cells were used for the development of dengue vaccine at the Walter Reed Army Institute of Research (WRAIR) in the USA. All four monovalent formulations elicited seroconversion in humans. The vaccine was well-tolerated, caused no clinically serious adverse events and induced the production of neutralizing antibodies to all four serotypes. Tetravalent formulations were prepared and evaluated in a monkey model. Challenge studies in rhesus monkeys demonstrated that most animals seroconverted after two doses of the vaccine. After virus challenge, viremia was measurable in 4 of 20 monkeys. In pilot studies in humans, three doses of tetravalent vaccine induced 50% and higher seroconversion to all four serotypes. The dissemination rates of WRAIR vaccine viruses in mosquitoes were low and it is unlikely that these viruses would be transmitted under natural conditions\(^6\). The next stages of the clinical trials are in progress.

**Chimeric vaccine**

Several research groups are successfully exploring infectious clone technology for the development of a dengue vaccine. The ChimeriVaxTM system, originally developed to construct JE vaccine, has now been applied to dengue viruses by Acambis in the USA. A chimeric YF-dengue type 2 virus (D2) was prepared, using a recombinant cDNA infectious clone of a YF vaccine strain (YF17D) as a backbone, into which the premembrane (PRM) and envelope (E) genes of dengue 2 virus were inserted\(^7\). YF vaccine was selected as a backbone because of its excellent safety record during a long period of practical use. All monkeys vaccinated with ChimeriVax-D2 virus developed neutralizing antibodies and were protected against challenge with a wild-type dengue-2 virus. The high replication efficiency, attenuation phenotype in animal models, immunogenicity and protective efficacy, and genomic stability of ChimeriVax-D2 justify it as a novel candidate vaccine for evaluation in humans. YF/dengue viruses for three other serotypes have been constructed and are undergoing laboratory analysis and evaluation in animal models.

Another approach is based on the use of a dengue type 4 mutant containing a deletion in non-coding regions as a genetic background for the construction of a dengue chimeric vaccine\(^8\). Viruses with deletion mutations are genetically more stable than the ones with point mutations and are less likely to revert to the genotype of the parent virus when propagated in vaccinees. On the basis of laboratory tests and work with a monkey model, some deletion mutants were defined as attenuated viruses. Phase 1 clinical trials of a 3' deletion mutant were carried out in adult humans. The results indicated that this dengue 4 deletion mutant was safe and immunogenic. It is planned to use this attenuated virus as the backbone for
the construction of chimeric dengue viruses of serotypes 1, 2 and 3. The ultimate aim is to develop a tetravalent vaccine.

Work at the Centers for Disease Control and Prevention in the USA showed that attenuation markers of dengue 2 vaccine strain PDK-53 were encoded by genetic loci outside the structural gene region\(^9\). On this basis, chimeric dengue type 2/type 1 viruses were constructed which contained the non-structural genes of PDK-53 and structural genes of the dengue 1 strain\(^10\). Chimeric virus retained the attenuation in vivo and in vitro markers and was immunogenic in mice, inducing the production of neutralizing antibodies against dengue 1. It is considered as a potential dengue 1 candidate vaccine. The results also suggest that the infectious clones from the PDK-53 vaccine are promising attenuated vectors for the development of chimeric flavivirus vaccines.

**DNA vaccines**

A candidate DNA vaccine expressing dengue virus type 1 PrM and E proteins was developed and used for the immunization of different kinds of monkeys\(^11,12\). The candidate vaccine induced the production of virus-neutralizing antibodies and gave partial protection against challenge with homologous dengue virus. Intramuscular immunization of rhesus macaques was more immunogenic than intradermal immunization. Another study focused on the construction of a dengue vaccine containing PrM and E genes of the Guinea C strain of dengue type 2 virus\(^13\). In immunized mice the candidate vaccine induced neutralizing antibody production and strong anamnestic responses to challenge. Further extensive preclinical and clinical trials are required before a decision can be made on the acceptability of DNA vaccine for practical use.

**Inactivated and subunit vaccines**

The success of inactivated flavivirus vaccines against JE in Japan and tick-borne encephalitis in Austria and Russia led to attempts to develop a killed dengue vaccine. However, early work in this area was unsuccessful because of difficulties in growing high titres of dengue virus in cell lines. It was recently shown that flaviviruses can grow to high titres in Vero cells\(^14\). Dengue virus type 2 was grown in Vero cells and, after inactivation, purification and concentration, was used for the immunization of laboratory animals\(^15\). The experimental vaccine induced the production of a protective level of antibodies in monkeys. This approach will probably allow the development of an effective inactivated dengue vaccine.

Recombinant DNA techniques provided the possibility of cloning specific genes encoding for protective antigens and of expressing them in other host cells, including E.coli, yeast and insect cell systems. This technology has been used by several researchers for the development of subunit vaccines. Recombinant E protein of dengue 2 virus, produced in a baculovirus vector system, induced neutralizing antibody production and partial protection of immunized monkeys\(^16\). Products from Drosophila cells appeared to be promising in
the early stages of testing in animals\textsuperscript{(15)}. Further efforts are required to increase the immunogenicity of subunit vaccines by incorporating them into adjuvants or other systems for stimulating immune responses.

**Vaccinia virus as vector for dengue vaccine**

The use of genetically-modified vaccinia virus as a vector for genes encoding flavivirus vaccine antigen could have broad application for the genetic engineering of viral vaccine. Modified vaccinia Ankara (MVA) vector with a restricted host range was developed for the construction of recombinants\textsuperscript{(17)}. The safety of this vector was demonstrated in a large number of volunteers. MVA and recombinants derived from this virus do not replicate efficiently in human and most other mammalian cells, and this character is genetically stable. Monkeys repeatedly immunized with MVA recombinant expressing dengue 2 E protein have virus-neutralizing antibodies and are fully protected against challenge with homotypic dengue virus\textsuperscript{(18)}. Work is planned on constructing MVA recombinants expressing immunogenic E protein of other dengue virus serotypes.

**WHO activity in the development of dengue vaccine**

WHO has designated the dengue viruses as a high-priority target for accelerated vaccine development. This work is conducted by a steering committee on dengue and JE vaccines, established in 1984. In the area of dengue vaccine, the main purpose of the steering committee is to promote and facilitate the development of candidate vaccines with a view to expediting their introduction in developing countries. This involves the evaluation of new biotechnological approaches, active participation in clinical trials of candidate vaccines, and the facilitation of vaccine introduction through the planning and assessment of low-cost vaccination schedules\textsuperscript{(19)}. The steering committee has supported some research projects that have led to the development of candidate vaccines now undergoing clinical evaluation.

In order to promote the evaluation of live attenuated vaccines in clinical trials, a group of WHO experts has been developing guidelines for the safety of dengue vaccine. These guidelines could help public health officials to make decisions about conducting dengue vaccine trials in their countries. They could also help researchers to arrive at technical decisions before designing trial protocols. The steering committee on dengue and JE vaccines supports research projects aimed at standardizing immunological methods, including the neutralization test for dengue viruses, to be used by laboratories involved in evaluating the immunogenicity of dengue vaccine.

**References**


