Dengue vaccines: Problems & prospects

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The extent of cumulative disease burden caused by dengue virus has attained an unprecedented level in recent times with sharp increase in the size of human population at risk. Dengue disease presents highly complex medical, economic and ecologic problems. The surge in publications on the development of dengue vaccines, taking advantage of new generation of biotechnology techniques indicates the profound interest and urgency in the scientific and medical communities in combating this disease. This review summarizes the importance of critical subjects like pathogenesis of dengue haemorrhagic fever and inadequacy of animal model that have adversely affected dengue vaccine development. Further, the remarkable progresses so far made in dengue vaccine research not only employing a diverse range of new strategies but also re-using old techniques to improve the existing vaccines, have been presented. The efficacy and safety of some of the new vaccine candidates have been evaluated and proven in human preclinical/clinical trials. Besides the technical advancement in vaccine development, vaccine safety and vaccine formulation have been examined.

Key words Attenuated vaccine - chimeric vaccine - dengue virus - DHF - DNA vaccine - pathogenesis - purified-inactivated vaccine - vaccine

Dengue virus (DV) infection is mostly asymptotic or produces a mild self-limiting acute febrile illness, dengue fever (DF), and a life-threatening severe illness, dengue haemorrhagic fever (DHF) with minor or major bleeding from different sites1. DHF has emerged as the most important arbovirus disease in man in the last three decades. It has been estimated that about 50 to 100 million cases of DF occur every year with about 250,000 to 500,000 cases of DHF2. During epidemics of dengue, attack rates population 40 to 90 per cent among susceptible of whom a very large proportion is of children3,4. The year 2001 witnessed unprecedented global dengue epidemic activity in the American hemisphere, the Pacific islands and continental Asia. During 2002, more than 30 Latin American countries reported over 10,00,000 DF cases with large number of DHF cases. This has been followed by extensive epidemics of DHF in several parts of India during 20034.

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DHF has been classified into four grades on the basis of the clinical presentation and laboratory findings; the mildest is grade I and the most severe is grade IV. The pathognomonic features of DHF are increased capillary permeability without morphological damage to the capillary endothelium, altered number and functions of leucocytes, increased haematocrit and thrombocytopenia. Extensive plasma leakage in various serous cavities of the body including the pleura, pericardium and peritoneal cavities in DHF grades III and IV may result in profound shock, the dengue shock syndrome (DSS). Today DHF affects most Asian countries and has become a leading cause of hospitalization and death among children in several of them. The risk factors for DHF are infestation with Aedes mosquito, hot and humid climate enhancing mosquito breeding, mosquito density, and presence of all the four serotype of the dengue virus with the secondary infection in the host, the water storage pattern in the houses, population density and large movement of people towards urban areas. At present, there is no specific therapy available for DHF. Appropriate symptomatic treatment has been successful in reducing the mortality of DHF. Mosquito control has been the only method of preventing DHF but is costly and often ineffective.

Is dengue vaccine possible?: In principle, an effective vaccine against DV is highly feasible because it causes only acute infection and the virus replication is effectively controlled after a short period of 3 to 7 days of viraemia. Further, the individuals who have recovered from DV infection, are immune to rechallenge with the same type but not to other types of DV. Studies conducted in mice have demonstrated that passive transfer of virus-specific antibodies protect them against subsequent DV challenge. Therefore, development of an effective vaccine against DV has been given a high priority.

Problems in development of dengue vaccine: More than sixty years after the discovery of the virus no effective vaccine is available. The problems are lack of understanding the pathogenesis of DHF and absence of an animal model for the dengue disease. Pre-existing heterotypic dengue antibody is a risk factor for DHF, therefore, an effective vaccine will have to be tetravalent and needs to prevent infection with all four DV serotypes. Natural DV infection induces long-lasting protective immunity only to the same serotype. A tetravalent formulation that retains the immunogenicity of all four serotypes has proven difficult, requiring the use of more complicated, multiple-dose immunization regimens.

A more significant obstacle is the current inability to predict whether candidate DV vaccines will be at all effective in preventing DHF. Studies of candidate vaccines have analyzed efficacy only in experimental animal models, none of which faithfully reproduces the DHF syndrome seen in humans. Therefore, selection of the most promising DV vaccine candidates relies on comparing vaccine-induced immune responses to a profile of protective immunity developed from natural DV infections.

Dengue virus antigens and immune response: DV genome is a single-stranded sense RNA which is translated as a single polyprotein that is cleaved by proteases of viral and host origin, to yield ten viral proteins including the C and M proteins, the E glycoprotein, and seven nonstructural (NS) proteins. The antibody and T cell responses to individual viral proteins are variable. Production of anti-E antibodies is the main response against DV that inhibits viral binding to cells, neutralizes viral infectivity in vitro, protects mice from DV challenge on passive transfer and shows a variable degree of cross-reactivity among the DV serotypes. The uptake of DV into monocytic cell lines and primary human monocytes in vitro is enhanced through binding of antibody to virus at non-neutralizing epitopes with cell surface Ig receptors or at concentrations below the neutralization endpoint. This is known as antibody-dependent enhancement of infection. NS1 expressed on the surface of the virus infected cells, is secreted into the circulation as a soluble multimer and is an important target of antibodies against DV. Antibodies against NS1 can trigger complement-mediated lysis of DV infected cells in vitro and protect mice from DV challenge. NS3 protein is the main antigen that stimulates CD4+ and CD8+ T cell response to DV, DV-reactive CD4+ and CD8+ T cells predominantly produce high levels of IFN-γ as well as TNF-α, TNF-β, and chemokines including macrophage inhibitory protein-1β upon interaction with DV infected antigen presenting cells,
and are efficient at lysis of DV infected cells *in vitro*[^14].[^15]. Contribution of the immune responses to the long-term protective immunity by natural primary DV infection is not fully known.

**Pathogenesis of dengue haemorrhagic fever:** Despite extensive studies, the pathogenesis of DHF is still not fully understood and has been a subject of controversy from the time the syndrome was first recognized. Opposing views have focused on the effect of viral and host factors on disease severity. Various mechanisms that have been considered include immune-complex disease, antibodies cross-reacting with vascular endothelium, enhancing antibodies, complement and its products, various soluble mediators including cytokines, selection of virulent strains and virus virulence, *etc*.[^4].[^6].[^16].[^19] (Table I).

**Cytokines in patients with dengue:** Cytokine secretion profile in patients with dengue disease has been summarized in Table II. The most significant finding reported for the first time on patients with DHF in India during 1996 was shift from the predominant helper T cell type 1 (Th1) response observed in cases of DF to the Th2-type in severe cases of DHF grade IV[^35]. As the severity of the illness increases the response shifts to Th2-type in majority of the cases with DHF grade IV[^35]. This has been confirmed in subsequent studies (Table II). IL-12 has a profound effect on the upregulation of Th1 cells while its absence shifts the balance towards Th2-type cytokines. IL-12 has been associated with clearance of virus, host recovery and protection in a large number of viral infections[^36]. Elevated levels of IL-12 are seen in the patients with milder dengue illness (DF) and complete absence in the patients with DHF grades III and IV[^47]. Thus, IL-12 may play a role in preventing the severe dengue disease by maintaining the Th1-type response. If this is true, IL-12 therapy may have profound beneficial effect on the outcome of severe dengue disease[^16]. Increased levels of IL-8 in the sera and IL-8-mRNA in the peripheral blood mononuclear cells (PBMC) are associated with the increasing severity of DHF and death. It has been suggested that presence of high levels of IL-8 may be a useful indicator of serious outcome of the dengue illness[^45]. Further, the severity of disease and the duration of illness are correlated with the level of transforming growth

| Table I. Proposed mechanisms for the development of dengue haemorrhagic fever (DHF) |
|---------------------------------------------|------------------|
| **Mechanisms**                              | **Effects**      |
| Antibody mediated                           |                  |
| Enhancement of infection                    | Increased cellular infection and viral load[^20].[^22] |
| Immune-complex formation                    | Complement activation[^23].[^25] |
| Cross-reactivity with endothelial cell and coagulation proteins | Bleeding[^26].[^28] |
| Cross-reactivity with endothelial cell and NS1 protein | Apoptosis, inflammatory activation[^18] |
| T-Lymphocytes                               |                  |
| Cytokines                                   | Increased capillary permeability[^16].[^29].[^31] |
| Bystander cell lysis                        | Liver injury[^15] |
| Virus virulence                             | Increased transmissibility and cellular infection[^33].[^34] Selection of virulent strains in humans and mosquitoes[^39] |

Superscript numerals denote reference numbers

<table>
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<th>Table II. Cytokines in patients with dengue infection</th>
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<td>Transforming growth factor-β</td>
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<td>Human cytotoxic factor</td>
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*Source:* Modified from Chaturvedi *et al.*[^16]; I, increased; MI, markedly increased; D, decreased; MD, markedly decreased; N, no change; DF, dengue fever; DHF, dengue haemorrhagic fever
factor-beta 1 (TGF-β1), i.e., the maximum levels of TGF-β1 are detected in patients with DHF grade IV.

Cytotoxic factor in patients with dengue: A unique cytokine, cytotoxic factor (CF) is produced by CD4+ T cells during dengue virus infection of mice (mCF) and man (hCF). The amino terminal sequence of mCF has no homology with any of the known proteins or cytokines. The hCF purified from the sera of DHF patients, when inoculated into mice increased capillary permeability and damaged the blood-brain barrier indicating its role in pathogenicity. mCF and hCF appear to be pathogenesis-related proteins, capable of reproducing DHF-like pathological lesions in mice, such as increased capillary permeability, cerebral oedema, and blood leukocyte changes.

Majority of the patients with dengue show the presence of hCF in their sera, with peak amounts in the most severe cases of DHF grade IV. Peripheral blood mononuclear cells of such patients cultured ex vivo show production of hCF by CD4+ T cells. The production of mCF/hCF precedes the clinical illness in mice and man. The DHF-like pathological lesions produced by mCF/hCF can be prevented by pre-treatment of mice with the anti-mCF antibodies. Further, active immunization of mice using mCF as antigen protects them against subsequent challenge with mCF. Challenge of such mice with a lethal intracerebral dose of DV results in death without appearance of clinical symptom of the disease. These studies suggested a vaccine strategy directed against the primary cause of the disease (the cytokine) rather than the infective agent as an effective vaccine against dengue is not yet available. In fact, similar strategies are now being successfully used in several diseases using anti-TNF-α antibody therapy. While the level of TNF-α is increased in a variety of conditions, mCF/hCF have the advantage of being present only in dengue. Further, highest levels of hCF autoantibodies are seen in sera of patients with mild illness (DF) while the levels decline sharply with the development of DHF, and the levels are lowest in patients with DHF grade IV. This suggests that higher levels of hCF autoantibodies protect the patients against the development of DHF and may be used as a prognostic indicator.

Proposed mechanism of pathogenesis in DHF: With the available data, we would like to propose a mechanism that may explain the pathogenesis of DHF (Fig.). Dengue virus replicates in macrophages and induces quickly the CD4+ T cells to produce a unique cytokine, hCF. hCF induces macrophages to produce free radicals, nitrite, reactive oxygen and peroxynitrite. The free radicals, besides killing the target cells by apoptosis also directly upregulate production of proinflammatory cytokines IL-1β, TNF-α, IL-8, and hydrogen peroxide in macrophages. The change in relative levels of IL-12 and TGF-β shifts a Th1-dominant response to a Th2-biased response resulting in an exacerbation of dengue disease and death of patients. The vascular permeability is increased due to the combined effect of histamine, free radicals, proinflammatory cytokines and the products of the complement pathway, etc. Thus, the key player

<table>
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appears to be hCF, but its activity is regulated by hCF-autoantibodies.

**Dengue vaccines:** There have been many efforts over the last six decades to produce a vaccine to combat DV infection; few have been able to meet the challenges posed by the unusual interplay between this virus and its human host. However, recent progress in molecular-based vaccine strategies, as well as a renewed commitment by the World Health Organization (WHO) to co-ordinate global efforts on vaccine development, finally provides hope that control of this serious disease may be at hand. Without effective antiviral drugs, vaccination offers the best chance of decreasing the incidence of these diseases, and live virus vaccines are the most promising and cost-effective. The efforts made in this direction are summarized in Table III.

**Live attenuated dengue vaccine:** The development of a live attenuated tetravalent dengue vaccine is currently the best strategy to obtain a vaccine against dengue viruses. WHO suggested the idea of a tetravalent dengue vaccine, which could confer antibody against all four types of DV, in 1978. Therefore, efforts to produce live attenuated dengue
vaccine using natural or chemical mutants and cloned in diploid foetal rhesus lung cell culture were made. The Thai group developed candidate live attenuated vaccines by attenuation through serial passages in primary dog kidney cell (PDKC) cultures. Dengue serotype 1, 2 and 4 viruses are developed in PDKC, whereas dengue serotype 3 is serially passaged in primary African green monkey kidney cells. Tissue culture passaged strain viruses are subjected to biological marker studies. Candidate vaccines have been tested as monovalent (single virus), bivalent (two viruses), trivalent (three viruses) and tetravalent (all four serotype viruses) vaccines in Thai volunteers. They are found to be safe and immunogenic in both adults and children. The live attenuated dengue 2 virus has also been tested in American volunteers and resulted in good immune response indistinguishable from those induced in Thai volunteers. The vaccines were recommended for human trials by a scientific steering committee appointed by the WHO. Sun et al. tested the four serotypes of monovalent live attenuated dengue virus vaccine candidates for reactogenicity and immunogenicity in 49 flavivirus non-immune adult human volunteers. The four monovalent candidates were then combined into a tetravalent formulation and given to another 10 volunteers. Neutralizing antibody seroconversion rates after a single-dose monovalent vaccination ranged from 53 to 100 per cent. Reactogenicity differed among the four serotype candidates with serotype 1 associated with the most vaccine related side effects. A second dose of monovalent vaccines at either 30 or 90 days is much less reactogenic but did not significantly increase seroconversion rates. Seroconversion rates in the 10 volunteers who received a single dose of tetravalent vaccine ranged from 30 to 70 per cent among the four serotypes. Similar to the monovalent vaccines, a second dose of the tetravalent vaccine after one month was less reactogenic and did not increase seroconversion. A third dose of the tetravalent vaccine after four months resulted in three of four volunteers with trivalent or tetravalent high titre neutralizing antibody responses. Phase I clinical trials have also been reported by Edelman et al. On the other hand, 8 of 69 (12%) healthy adult volunteers vaccinated with monovalent live-attenuated dengue vaccine candidates showed atypical antibody responses, with depressed IgM:IgG antibody ratios and induction of high titre haemagglutination-inhibiting and neutralizing antibodies to all four DV serotypes. The safety and immunogenicity of tetravalent live-attenuated dengue vaccines after a three dose vaccination series were evaluated in Thai children. No serious adverse event related to the vaccines occurred. Most children experienced mild to moderate fever, rash, headache and myalgia occurring within 12 days after dose 1 and generally lasting 3 days or less. One subject had a dengue-like fever for one week. Reactogenicity was minimal after doses 2 and 3. Transient mild variations in liver enzymes and haematologic indices were noted mainly after dose 1. This study demonstrates a moderate although improvable reactogenicity and high seroconversion rates against the four serotypes of dengue after a three-dose schedule of tetravalent live-attenuated dengue vaccine in children.

Gwinn et al. reported an antigen-specific Th1 type cell response to vaccination using live-attenuated dengue virus. The findings of Guy et al. on sera from Thai children immunized with a live-attenuated tetravalent DV vaccine or from naturally infected age-matched site-control subjects, support the use of live dengue vaccines and protocols that induce broad serotype-specific neutralizing antibody responses, but they also suggest that clinically relevant immune enhancement may not be likely if this is not uniformly achieved after the first immunization. Zhang et al. reported the possibility of attenuating DV infection using adeno-associated virus (AAV)-encoded short interfering RNAs (siRNA) in Vero cells and human dendritic cells. The replication of attenuated DV can be further decreased by introduction of mutation into the non-structural gene.

Chimeric dengue vaccine: Recent advances in recombinant DNA technology have made it possible to explore a novel approach for developing live attenuated viral vaccines. Full-length cDNA clones allow construction of infectious virus bearing attenuating mutations or deletions incorporated in the viral genome. It is also possible to create chimeric viruses in which the structural protein genes for the target antigens of a virus are replaced by the corresponding genes of another virus. By combining these molecular techniques, a chimeric virus is
constructed with the required attenuation phenotype and expression of the target antigens. Robbert et al. have constructed a chimeric yellow fever/dengue virus (ChimeriVax-DEN), which expressed the premembrane (prM) and E genes from DV type 2 (DV-2) in an YF virus (YFV-17D) genetic background. Immunization of BALB/c mice with this chimeric virus induced a CD8 T-cell response specific for the DV-2 prM and E proteins. This response protected YF/DV-immunized mice against lethal dengue encephalitis. The strategy of creating chimeric flaviviruses opens new avenues for dengue virus vaccine development. Guirakhoo et al. constructed ChimeriVax-DEN representing DV serotypes 1 to 4 by electroporation of Vero cells with RNA transcripts prepared from viral cDNA. Progeny viruses are plaque purified to produce the vaccine. Preclinical studies demonstrated that the vaccine candidates are replication competent, genetically stable and do not become more neurovirulent upon 20 passages in Vero cells. The safety of the recombinant DV tetravalent vaccine has been demonstrated in a formal neurovirulence test, as well as its protective efficacy in a monkey challenge model. Further, ChimeriVax-DEN viruses infected mosquitoes poorly via an infectious blood meal compared with wild DV. Therefore, it is unlikely that a mosquito feeding on a viremic vaccine would become infected with the chimeric vaccine viruses. Encouraging results from preclinical and clinical studies have shown that several chimeric flavivirus vaccines have the safety profile and satisfactory immunogenicity and protective efficacy to warrant further evaluation in humans. The chimeric flavivirus strategy has led to the rapid development of novel live-attenuated vaccines against dengue, tick-born encephalitis (TBE), Japanese encephalitis (JE), and West Nile viruses. A chimeric vaccine based on a recombinant yellow fever vaccine for the potential prevention of dengue virus infection (ChimeriVax-DEN) is undergoing phase I clinical trials.

**DNA dengue vaccines:** DNA vaccine technology, in which plasmids expressing appropriate viral antigens are used for immunization has been shown to induce an immune response in animal models against a number of different viruses, including several flaviviruses. All of them have been reported to induce antibodies in mice and provide full or partial protection from live virus challenge. The first attempt to use DNA in the development of potential DV-2 vaccine was made by Kochel et al. They cloned PreM and the E genes into different eukaryotic plasmid expression vectors and inoculated intradermally in BALB/c mice resulting in development of anti-dengue antibodies which neutralized DV-2 in vitro. In a lethal mouse intracerebral challenge model, 60 per cent of the mice immunized survived the challenge. The study by Ocazionez Jimenez and Lopes da Fonseca corroborates with the hypothesis that pre-membrane protein (prM) is important for the processing of the E glycoprotein and should be incorporated on candidate vaccines engineered by recombinant DNA technology. Putnak et al. have reported induction of neutralizing antibodies to DV-2 and antigen-specific cytotoxic T lymphocyte responses in mice by a similar vaccine. They further suggested that in rhesus macaques a regimen consisting of two 1 µg doses of DNA can confer satisfactory protection at one month, but not at seven months, after vaccination. Long-term protection following DNA vaccination may require re-vaccination and higher doses of DNA. A similar DV-1 DNA vaccine elicits virus neutralizing antibodies in rhesus and Aotus monkeys, and the primates are partially protected from viraemia upon challenge. Raviprakash et al. evaluated strategies to increase the neutralizing antibody levels and subsequent protection from virus challenge: (i) co-immunization with a plasmid expressing Aotus GM-CSF gene; (ii) co-immunization with a plasmid containing human immunostimulatory sequences (ISS); (iii) co-immunization with both the GM-CSF gene and ISS; and (iv) delivery of vaccine using the needle-free Biojector system. Vaccination with the mixed formulation by either needle injection or Biojector, led to neutralizing antibody titres that are stable for up to 6 months after vaccination and 87 per cent receiving this formulation are completely protected from viraemia when challenged 1 and 6 months after vaccination, respectively.

The highest antibody responses are noted when the modified construct is co-injected with plasmid expressing the GM-CSF gene. The NS1-DNA-induced protection can be further augmented by co-injection of plasmid encoding interleukin-12 (IL-12), suggesting an effector role of Th1 immunity against...
DV infection. Further studies underscore the importance of major histocompatibility (MHC) class II presentation of DNA-encoded DV E protein for production of neutralizing antibodies. The results of Wu et al. suggest the potential of NS1-DNA vaccine against DV infection, and indicate both NS1-specific humoral and cellular immune responses contribute to the protection. DNA immunization has also been used to produce monoclonal antibodies against NS1 protein of DV. Konishi et al. have used combination vaccine consisting of DNA from DV-1, DV-2 and Japanese encephalitis virus to study response in mice.

Inactivated dengue vaccines: In a recent review, the problems and prospects of inactivated flavivirus vaccines have been discussed in detail, therefore, only salient points have been summarized here. Attempts to produce inactivated dengue vaccine have been made for more than sixty years but the obstacle was poor yield of the virus with the commonly used techniques. With the use of foetal rhesus lung diploid cell cultures and Vero cell cultures it is possible now to grow DV in high titres. Candidate vaccines have been prepared from whole virus particle or recombinant subunit proteins of dengue virus.

(i) Whole virus inactivated dengue vaccine: Putnak et al. grew DV-2 in Vero cell culture, purified on sucrose gradients and inactivated with 0.05 per cent formalin at 22°C. After inactivation, the virus retains its antigenicity and is immunogenic in mice and rhesus monkeys, in which it elicits high titres of DV-2-neutralizing antibody, which completely protect animals against challenge with live, virulent virus. A new DV-2 vaccine produced in serum-free medium has proven satisfactory in initial pre-clinical safety evaluations and was scheduled for phase I clinical trials.

(ii) Recombinant subunit dengue vaccine: Advancements in the molecular biology techniques have facilitated the development of recombinant subunit vaccines for different viruses. Majority of efforts have been made to get recombinant E, while some have tried NS proteins of DV. For good immune response it is essential that the recombinant protein be secreted extracellularly. This has been made possible by expressing DV-2 E protein together with prM or as a fusion with hepatitis B surface antigen. Immunization of mice with DV-2 NS1, purified from lysates of infected Vero cells by immunoaffinity chromatography provides significant protection against intracerebral challenge. On the other hand, mice inoculated with purified recombinant DV-2 NS1 protein produce antibodies against NS1 but are not neutralizing and the animals are not protected against a lethal viral challenge.

Precautions in dengue vaccine formulations: Although the antibody-dependent enhancement phenomenon is well documented in vitro, its importance in vivo remains to be determined. Nevertheless, the potential role of cross-reactive, non-neutralizing antibodies in DHF has obvious implications for vaccine design: any effective dengue vaccine should induce neutralizing antibodies and/or T-cell immunity against all four serotypes. It has been reported that development of DHF is strain specific and may be correlated with specific amino acid sequences in the E protein. Cologna et al. have used human dendritic cells and Aedes aegypti mosquitoes for measuring differences in virus replication that correlate with the potential to cause haemorrhagic dengue and increased virus transmission. They observed that the Southeast Asian genotype DV-2 strains causing DHF epidemics can outcompete the
American genotype viruses that cause DF only. This fact implies that Southeast Asian genotype viruses will continue to displace other viruses, causing more haemorrhagic dengue epidemics. These reports are of critical importance for vaccine design, since it identifies the sequences that should probably be avoided. Safety issues with the live flavivirus vaccines need to be recognised and addressed. The theoretical possibility of recombination between a vaccine strain and wild-type virus resulting in a new virus with potentially undesirable properties can never be entirely dismissed, but steps can be taken to minimize risk. The development of non-live DV vaccines could be an alternative.

**Conclusions**

Pre-existing heterotypic dengue virus (DV) antibody is a risk factor for DHF, therefore, an effective vaccine will have to be tetravalent (all the four types of DV). Candidate attenuated vaccine viruses have been evaluated in phase I and II trials in Thailand, and a tetravalent formulation is currently undergoing repeat phase I and II trials. Advances have also been made with second generation recombinant dengue vaccines. A cDNA infectious clone of the DV-2 PDK-53 vaccine candidate virus has been constructed, and work is in progress to construct chimaeric viruses by inserting the capsid, pre-membrane, and envelope genes of DV 1, 3, and 4 into

### Table IV. Recombinant candidate dengue vaccines

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<tr>
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<th>Antibody response</th>
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<tr>
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ab, Antibody; C, structural C protein; CHO, Chinese hamster kidney; E, envelope glycoprotein; M, structural membrane protein; Nab, neutralizing antibody; NA, not known; NHP, non-human primate; PrM, pre-membrane protein; sf9, *Spodoptera frugiperda* cells; NS, non-structural protein
the DV-2 PDK-53 backbone. These recombinants, through genetic manipulation, may be made to replicate faster, be more immunogenic, and safer. However, an effective, safe and affordable vaccine is not an immediate prospect.

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