DEN2 NGC and chimeric DEN4/DEN2 viruses developed encephalitis and died. There was, however, a three- to five-day delay in death caused by the chimera, while both the DEN1 WP and DEN4/DEN1 viruses were not neurovirulent. Later, DEN4/DEN2 chimeras containing the prM-E cassette or only NS1 gene from DEN2 in place of the corresponding genes in the DEN4 backbone were successfully generated, while attempts to produce a chimera containing DEN2 C-prM-E-NS1 genes failed (45). A DEN4/DEN3 chimera containing the C-prM-E genes from a wild-type DEN3 strain CH53489 was also obtained (46). These studies demonstrated the possibility of engineering viable intertypic DEN chimeras and also yielded valuable information on genetic determinants of neurovirulence in mice (47).

Subcutaneous (SC) inoculation of rhesus monkeys at a dose of 3×10^5 pfu of either the DEN4/DEN1$_{\text{C-prM-E}}$ or DEN4/DEN2$_{\text{prM-E}}$ chimera induced detectable, short-lived viremias. Immunization resulted in high titers of homologous DEN type-specific neutralizing antibodies (1:640 to 1:1280), which were similar to titers observed in control animals inoculated with the DEN1 and DEN2 parents.

Challenge of monkeys immunized with the DEN4/DEN1$_{\text{C-prM-E}}$ or DEN4/DEN2$_{\text{prM-E}}$ chimeras at 66 days post-immunization with the corresponding wild-type DEN viruses demonstrated no viremia in the majority of immunized animals, while high titer viremias were observed in all unimmunized controls. Similarly, monkeys immunized with a mixture of the two chimeras (DEN4/DEN1$_{\text{C-prM-E}}$ and DEN4/DEN2$_{\text{prM-E}}$) developed high titers of both DEN1- and DEN2-specific neutralizing antibodies (generally 1:320 to 1:640). The animals were solidly protected from challenge with both wild-type DEN1 and DEN2, even despite the fact that the DEN4/DEN2 chimera clearly outgrew the DEN4/DEN1 chimera in the doubly immunized animals as evidenced by the analysis of post-immunization viremias (48). These data suggested that developing a tetravalent DEN vaccine composed of chimeric viruses based on the genetic background of one flavivirus is possible. The DEN4 virus used in these studies (which would be also the DEN4 component in a tetravalent vaccine formulation, along with three DEN4-based chimeras) is pathogenic for humans and transmissible by mosquitoes.

The DEN4 backbone–based approach to DEN vaccine has been pursued by scientists at the NIH and the Food and Drug Administration (FDA) (49–52). To increase safety in humans and decrease the rate of replication in mosquitoes, an attempt was made to attenuate the DEN4 virus by introducing large deletions in its 3′ untranslated region (53). A DEN4 variant with a 30-nt deletion (designated Δ30) was chosen for evaluation in humans (51), and it was proposed that a tetravalent vaccine could be made on the basis of wild-type DEN strains containing this deletion or intertypic chimeras constructed using the DEN4Δ30 backbone. Some of the published data suggest that the Δ30 deletion may not be sufficiently attenuating, and additional modification of the backbone is necessary (50,54). The DEN-4Δ30 virus was associated with mild adverse reactions in a high percentage of vaccinees, even when the vaccine was given at a low dose (e.g., rash in 75% of recipients of a 10-pfu dose), and occasional elevations in blood level of alanine aminotransferase at higher doses suggested replication of the virus in the liver (52). In addition, the Δ30 mutation did not attenuate DEN3 virus in monkeys, and did not reduce replication of DEN3 and DEN1 in mosquitoes (49,55). New vaccine candidates are being further developed and the possibility of using both chimeric and non-chimeric viruses in tetravalent mixtures delivered in one- or two-dose regimens is being explored (56). Attenuated DEN1Δ30 and a chimeric DEN4Δ30/DEN2 variant have been tested in DEN-naïve adult volunteers at a dose of 10^3 pfu. Notably, most vaccinees seroconverted to DEN1 or DEN2, respectively, and maintained significant antibody titers throughout the six-month trial duration demonstrating high durability of immune response (57,58). A highly attenuated DEN4Δ30/DEN1 candidate is also available and has been tested in rhesus monkeys (59).

Another promising chimeric approach to tetravalent DEN vaccine has been developed by scientists at the U.S. Centers for Disease Control and Prevention (CDC). Their chimeras are based on the backbone of the attenuated DEN2 PDK-53 virus, which is more attenuated and safer as a chimeric vaccine vector than the DEN4 backbone described above. The PDK-53 DEN2 virus strain was originally developed at Mahidol University (Bangkok, Thailand) by multiple passages of a wild-type DEN2 virus isolate in primary dog kidney cells. This chimeric method had been facilitated by the finding that all attenuating determinants in DEN2 PDK-53 virus map to the backbone, outside the prM-E genes, which can be replaced with heterologous DEN counterparts. PDK-53-based chimeras have been tested in mice and shown to be highly attenuated and immunogenic (7,60). PDK-53 DEN2/DEN1 vaccine variants against DEN1 induced DEN1-specific neutralizing antibodies in cynomolgus monkeys, without viremia. Most immunized monkeys were protected from wild-type DEN1 virus challenge (as well as DEN2) as judged by the analysis of post-challenge viremia (61).

Chimeras Between Unrelated Flaviviruses

The successful construction of intertypic DEN chimeras described above stimulated experimentation to develop chimeras between unrelated flaviviruses from different serocomplexes. The first report of a viable chimera between two genetically distant flaviviruses, a mosquito-borne DEN4 and a TBE virus, was published by Pletnev and coworkers in 1992 (62). The DEN4 backbone described above (from the DEN4 wild-type strain 814669) was used in these experiments. The DEN4/TBE chimera contained the prM-E genes of the Far Eastern strain Sofjin of TBE virus. The chimera grew efficiently in simian LLC-MK$_2$ cells but not in mosquito C6/36 cells, in contrast to the parental DEN4, which grows more efficiently in mosquito cells than in simian cells. Interestingly another chimeric variant, containing the TBE C-prM-E genes, was also viable but did not replicate efficiently compared with the prM-E chimera. This may be due to a number of problems that include inefficient encapsidation of the hybrid genomic RNA by the TBE-specific C protein, disruption of viral RNA cyclization essential for viral RNA synthesis (63) and inefficient cleavage at the C-terminus of the TBE-specific C protein by the DEN4-specific viral protease that generates the mature form of C (C$_{\text{virion}}$) (Fig. 1B) (64–66). Attempts to generate other chimeras containing TBE-specific prM-E-NS1 or E-NS1 cassettes, or singly C, E, or NS1 genes, did not result in viable viruses (62). As discussed above, a DEN4/DEN2$_{\text{NS1}}$ chimera was viable (45). Thus, in contrast to closely related DEN types, NS1 is not easily interchangeable between distant flaviviruses, possibly because NS1 is a component of viral RNA polymerase (11), which could be highly constrained for structural compatibility of all participating proteins.

Even though the efficiently replicating DEN4/TBE$_{\text{prM-E}}$ chimera contained the envelope of an encephalitogenic TBE