ChimeriVax-DEN

The first viable YF 17D/DEN chimera (YF 17D/DEN2, subsequently designated ChimeriVax-DEN2) was constructed by Chambers' group, by insertion of the prM-E genes from a wild-type strain of DEN 2 PUO-218 isolated from a patient in Thailand, and shown to be highly attenuated and immunogenic in mice and rhesus monkeys at Sanofi Pasteur (97). Interestingly, initial attempts to produce a similar chimera by Caufour et al. failed (98). Close examination of the two cloning strategies revealed that Caufour et al. initially followed the approach used in the construction of DEN4/TBE chimera (62). Specifically, they attempted to fuse the 5′ end of the DEN2 prM gene with the 3′ end of the YF C gene at the $C_{virion}/C_{intracellular}$ viral protease cleavage site and thus the transmembrane signal peptide for prM was DEN2-specific, whereas ChimeriVax-DEN2 virus was engineered to contain the YF-specific signal sequence (Fig. 1B). Because YF and TBE viruses both contain a 20 amino acid-long signal while DEN2 and DEN4 viruses have a shorter 14-amino acid signal, these results indicated that the length of the signal peptide is important for chimera viability. It appears that in DEN4-based chimeras, the short DEN4-specific signal for prM can be replaced with a longer one from another flavivirus, resulting in a viable chimera [although specific amino acid residues in the vicinity of the viral protease and signalase cleavage sites flanking the signal peptide play a role also, as shown for DEN4/WN (71)], whereas the long YF-specific signal needs to be retained in YF 17D-based chimeras. Consistent with this view, when the long YF-specific signal was replaced with the short DEN2-specific signal in ChimeriVax-DEN2, viability was lost (Miller and Arroyo, Sanofi Pasteur unpublished data), and Caufour et al. obtained a viable YF 17D/DEN2 construct using the YF-specific signal (98). The likely explanation for these observations is that the source (length) of the prM signal peptide in these flavivirus chimeras affects the coordinated fashion of cleavages at the flanking viral protease and signalase sites that is known to be critical for flavivirus replication (64–66). In this regard, it is interesting that a long WN-specific signal was found to be required for viability of PDK-53 DEN2/WN chimera (74).

Three other YF 17D/DEN virus chimeras, ChimeriVax-DEN1, ChimeriVax-DEN3, and ChimeriVax-DEN4, were constructed at Sanofi Pasteur (77,99–101). They contain the prM-E genes from wild-type DEN1 PUO 359 (Thailand, 1980), DEN3 PaH881/88 (Thailand, 1988), DEN4 1228 (Indonesia, 1978) strains, respectively. Similar to ChimeriVax-JE, the ChimeriVax-DEN chimeras are avirulent for young mice, and significantly less neurovirulent in suckling mice than the YF 17D virus. These chimeric viruses grow to titers of approximately 10^7 pfu/mL in Vero cells used for GMP manufacturing. The viruses are highly genetically and phenotypically stable, as very few mutations accumulated during serial passage in cell culture, and there was no increase in mouse neurovirulence after 13 to 18 passages in Vero cells. In rhesus monkeys, the four DEN chimeras administered by the SC route as mono- or tetravalent formulations produced low, brief viremias with peak titers of approximately 2 $\log_{10}$ pfu/mL. In comparison, viremias of the parental wild-type DEN viruses were as high as 4.9 $\log_{10}$ pfu/mL. Strong neutralizing antibody responses of the expected type specificities were induced in sera of immunized animals (99–101). Graded doses of the DEN2 chimera ranging from 2 to 5 $\log_{10}$ pfu/dose were tested and resulted in similar levels of DEN2-neutralizing antibody titers of approximately 1:320 on day 30, illustrating high immunogenicity of these viruses. Subsequent SC challenge with 5.0 $\log_{10}$ pfu of a wild-type DEN2 resulted in no detectable viremia of challenging virus in any of the immunized animals (97).

Testing of tetravalent mixtures of ChimeriVax-DEN1-4 in rhesus monkeys indicated that an appropriate formulation of the components must be determined to achieve uniform antibody responses against all four serotypes. When monkeys were given a mixture of 4.7 $\log_{10}$ pfu of each chimera (99), the ChimeriVax-DEN2 virus induced a higher viremia than the other three. Whereas monkeys seroconverted to all four serotypes, the anti-DEN2 neutralizing antibody titers were higher (1:142, 1:905, 1:127, and 1:71 against DEN1–4, respectively, on day 180). Unequal rates of virus replication upon simultaneous inoculation have been observed previously with other DEN vaccine candidates in both monkeys and human volunteers (48,102). A second inoculation with the same ChimeriVax-DEN1-4 tetravalent formulation resulted in no detectable viremia of any virus, indicating that primary immunization was protective, and the antibody titers became more uniform (1:640, 1:1810, 1:452, and 1:359, respectively). Thus, a more uniform immunity to all four serotypes could be attained by a two-dose vaccination. Importantly, high titers of DEN-specific neutralizing antibodies were induced in both YF-immune and naïve monkeys to confirm that anti-vector immunity is not a concern (99,100). In another experiment, the amount of ChimeriVax-DEN2 was reduced to 3 $\log_{10}$ pfu, to reduce dominance of this candidate, while doses of the other three viruses were 5 $\log_{10}$ pfu each (100). The dose adjustment resulted in a more balanced immune response to DEN1, 2, and 3, but somewhat higher against DEN4 (mean titers of 1:360, 1:400, 1:250, and 1:1400, respectively). Thus, the immune response to the tetravalent vaccine can be regulated by adjusting proportions of its components. Administration of a second tetravalent dose two months after the primary immunization increased antibody titers to all four serotypes. Antibodies in sera of the immunized animals efficiently neutralized various wild-type DEN strains from different geographic regions (100).

Plaque-purified cGMP vaccine lots of ChimeriVax-DEN1-4 vaccine candidates for human clinical trials were manufactured at Acambis and Sanofi Pasteur, and tetravalent mixtures were examined for neurovirulence and protective efficacy in cynomolgus monkeys (77). Brain lesions produced by a 5,5,5,5 ($\log_{10}$ pfu for each of the four components) tetravalent formulation after IC inoculation were significantly less severe than those observed with YF 17D (YF-VAX), and there were no nonneural tissue abnormalities. The immunogenicity and protective efficacy of four different tetravalent formulations (5,5,5,5, 5,5,5,3, 3,5,5,3, and 3,3,3,3 $\log_{10}$ pfu of each of the four respective serotypes) were evaluated after a single-dose SC vaccination. Most of the monkeys in the groups, and all monkeys that received equal-dose mixtures (5,5,5,5 and 3,3,3,3) seroconverted against all four DEN virus serotypes. All monkeys were protected from challenge with wild-type DEN1-4 viruses at six months post-vaccination, as evidenced by lack of post-challenge viremia, with the exception of one animal from the 5,5,5,3 and another animal from the 3,5,5,3 groups challenged with DEN1 and DEN4 viruses, respectively. Thus, the 5,5,5,5 and 3,3,3,3 formulations of these plaque-purified vaccine viruses were 100% protective. These results demonstrated safety of a recombinant tetravalent DEN vaccine in a formal neurovirulence test and its complete protective efficacy in a monkey challenge model.