antibodies recognizing the conserved fusion peptide of the E protein were recently shown to significantly increase replication of DEN viruses in Fc-receptor bearing cells in vitro and to increase viremia in monkeys, and modifications in the antibody Fc region could abrogate the ADE activity (32). This observation could lead to a new antibody-based therapy of DEN disease. Both virus-specified and host-related factors may influence the severity of the disease, because only approximately 3% of persons with secondary infections develop DHF. Alternatively, DHF may be caused by particularly virulent strains (33,34), and it has been suggested that anti-NS1 antibodies may cross-react with fibrinogen, thrombocytes, and endothelial cells triggering hemorrhage (35). It is generally accepted that a DEN vaccine must be tetravalent, capable of inducing a robust protective immunity against all four serotypes simultaneously, as well as long-term memory and persistence of neutralizing antibodies. Despite more than 60 years of extensive research efforts, no licensed DEN vaccine is yet available, although several promising vaccine candidates, including empirically attenuated DEN viruses, are in preclinical and clinical development (5–8).

### West Nile Virus

Since the unprecedented introduction of WN virus in 1999 from the Middle East to the New York City area (36), the virus has rapidly spread through North America, the Caribbean, and Mexico, and recently reached continental South America. WN virus is endemic in Africa, the Indian subcontinent, parts of Europe, Southern Russia, Central Asia, and the Middle East. The human disease varies from mild DEN-like illness to fatal meningoencephalitis, with the most severe illness occurring in the elderly (2,3). In the United States, disease incidence peaked in 2003, with 9862 reported cases, approximately one-third of which were accompanied by neurological symptoms, and 264 deaths. In 2004 to 2008 the incidence declined approximately three-fold, with 100 to 177 deaths annually, and it was hoped that the virus was genetically adapting to the new environment and becoming less virulent (37). Surveillance data from the 2007 WN season in the United States recorded another year with substantial disease burden as 3630 cases and 124 deaths were reported (38). As of October 7, 2008, 1030 cases and 24 deaths were reported in the United States for 2008. The emergence of WN in North America has spurred extensive interest in the development of human and veterinary vaccines. While there is still no human vaccine available, in 2006 Intervet Inc. (Millsboro, Delaware, U.S.) received approval of the first live attenuated chimeric single-dose vaccine for horses based on the ChimeriVax-WN01 virus (see below).

### Yellow Fever

YF virus, the prototype member of the Flavivirus genus, was first clinically recognized in the 17th century and remained one of the most dreaded diseases in tropical Africa and South America until the 20th century when an effective vaccine was developed. The symptoms of this lethal hemorrhagic fever transmitted to humans by *Aedes* mosquitoes include fever, hepatic, renal, and myocardial injury, hemorrhage, prostration, and shock. Today, because of incomplete vaccination coverage and mosquito reinfestation, YF still affects approximately 200,000 persons a year, and continues to be a threat to travelers (39). Wild-type YF virus, strain Asibi, was first isolated in 1927 by inoculation of a rhesus monkey with blood from a patient in Ghana. In 1937, Theiler and Smith reported successful attenuation of this virus by multiple passages in mouse and chick embryo tissues that yielded the 17D vaccine strain (40). In the approximately 70 years since its development, the 17D vaccine has been administered to over 400 million people with a remarkable history of safety and efficacy. The 17D vaccine is currently manufactured in several countries (France, United States, Russia, Switzerland, Senegal, Colombia, China, and India) in embryonated chicken eggs under standards established by WHO, and a sub-strain of 17D (called 17DD) is produced in Brazil. The vaccine is well tolerated, with few, usually mild, side effects such as injection site pain, redness, headache, etc. After vaccination, a low viremia is detectable during the first few days, not exceeding 2 $\log_{10}$ pfu/mL. Because of a low viremia in vaccinated individuals and the fact that, in contrast to wild-type virus, the 17D virus does not replicate in mosquitoes, vaccination cannot lead to dissemination of 17D. Vaccination is contraindicated in persons with immune deficiency disorders or those taking immunosuppressive medications. Owing to the vulnerability of infants, the vaccine is not recommended in children younger than nine months. Except when disease is epidemic, pregnancy is generally regarded as another contraindication, as congenital infection has been shown to occur at rate of 1% to 2%, although this has not clearly been associated with any harm to the fetus (39). The period of onset of immunity is short. Ninety percent of vaccinees develop protective levels of YF-neutralizing antibodies by day 10, and 99% by day 30 after vaccination. Immunity appears to be lifelong after a single dose; therefore, the 17D vaccine has been regarded as one of the strongest immunogens ever developed. This is probably due to the fact that 17D virus infects dendritic cells, the main antigen-presenting cells, and stimulates strong polyvalent immune responses through activation of multiple Toll-like receptors (41,42). All the above features of the 17D vaccine validate the use of YF 17D virus in the construction of novel, genetically engineered vaccines against other flavivirus diseases.

## FLAVIVIRUS CHIMERAS NOT BASED ON THE YF 17D BACKBONE

### Dengue Intertypic Chimeras

The introduction of methods of reverse genetics, or infectious clone technology, opened a new chapter in RNA virus research. An infectious clone is a DNA copy of a viral RNA genome, which is stably cloned (most frequently in bacteria), and can be easily manipulated in vitro. To initiate virus replication, the cDNA template is converted to RNA by in vitro transcription, and appropriate substrate cells are transfected with the RNA transcripts. Alternatively, cells are directly transfected with appropriately designed plasmid DNA. The first flavivirus infectious clone was reported in 1989, for YF 17D virus (43). Since then, infectious clones have been developed for many disease-causing flaviviruses, with the exception of DEN type 3 (DEN3). Infectious clones of flavivirus genomes are now used as tools to construct genetically engineered vaccines including chimeric flavivirus vaccine candidates.

The construction of the first viable flavivirus chimera was reported in 1991 by Bray and Lai of the National Institutes of Health (NIH), who replaced the entire structural region, the C-prM-E genes, in the infectious clone of DEN4 (wild-type strain 814669) with the corresponding C-prM-E cassettes from DEN1 (Western Pacific strain, WP) and DEN2 [New Guinea C (NGC), a laboratory strain neurovirulent for mice] (44). Three-day-old suckling mice inoculated intracerebrally (IC) with both the