Chimeric Vaccines Against Japanese Encephalitis, Dengue, and West Nile

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INTRODUCTION

Japanese encephalitis (JE), dengue (DEN), and West Nile (WN) viruses are among the most important human pathogens in the *Flavivirus* genus of the *Flaviviridae* family. This group of small enveloped RNA viruses includes approximately 70 members, 38 of which have been associated with human illnesses. In addition to the *Flavivirus* genus, the *Flaviviridae* family also includes the *Pestivirus* genus containing several veterinary pathogens that have a worldwide economic impact, such as bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV), the *Hepacivirus* genus that includes hepatitis C virus (HCV), an important human pathogen, and several recently identified hepatitis GB viruses not linked to human disease. Although currently grouped within the *Flaviviridae* family, pestiviruses and hepaciviruses differ significantly from representatives of the *Flavivirus* genus in terms of their life cycle, genome organization, processing of viral proteins, etc. (1). Therefore, the chimeric vaccine development approaches discussed in this chapter may not be easily applicable to viruses other than those in the *Flavivirus* genus. This chapter uses the term ''flavivirus'' to refer to members of the *Flavivirus* genus only.

With a few exceptions, flaviviruses are arthropod-borne viruses (arboviruses) transmitted by mosquitoes and ticks. On the basis of antigenic cross-reactivity and genome sequence similarity, flaviviruses are grouped into four distinct complexes (Table 1): the yellow fever (YF) complex containing YF virus as its sole member; the JE complex [JE, WN, St. Louis encephalitis (SLE), Kunjin (KUN), Murray Valley encephalitis (MVE) viruses, etc.]; the DEN complex that includes the four serotypes of DEN viruses (DEN types 1–4); and the tick-borne encephalitis (TBE) complex [TBE, Kyasanur forest disease (KFD), Langat (LGT), Powassan, louping ill viruses, etc.] (2). There are no antiviral drugs for the treatment of flavivirus infections, although development of new therapeutics has recently accelerated (3), and vector eradication programs have been inefficient in controlling YF, DEN, and the flaviviral encephalitides (4). Therefore, vaccination of people that live in or travel to endemic areas is the most effective means of protection against these diseases. There are currently no licensed vaccines against DEN and WN encephalitis. The vaccines that are currently available against YF, JE, and TBE, although efficacious, may still benefit from improvements in terms of their safety, efficacy, manufacturing cost, and/or use of acceptable cell substrates by implementation of new molecular biology and cell biology technologies. Several new molecular approaches (recombinant subunit vaccines, DNA vaccines, viruses attenuated using various genetic manipulations) as well as the classical approaches (killed-virus and empirically attenuated vaccine strains) are currently being explored to create DEN, WN, and new JE vaccines (5–8). This chapter will focus on the construction of live chimeric vaccines, particularly those generated using the ChimeriVax® technology developed by Sanofi Pasteur.

FLAVIVIRUS STRUCTURE AND REPLICATION

Flavivirions are spherical particles approximately 50 nm in diameter, the structure of which has been defined in detail with X-ray crystallography and cryoelectron microscopy (1) (Fig. 1A). The genome is a single-stranded RNA molecule of positive polarity of about 11,000 nucleotides (nt) in length. It contains a long open reading frame (ORF) flanked by 5′ and 3′ untranslated terminal regions (UTRs), approximately 120 and 500 nt in length, respectively. The ORF encodes a polyprotein precursor that is cleaved co- and posttranslationally, resulting in individual viral proteins. The virus proteins are encoded in the order: C-prM/M-E-NS1-NS2A/2B-NS3-NS4A/4B-NS5, where C (core), prM/M (pre-membrane/membrane), and E (envelope) are the structural proteins, that is, the components of viral particles, and NS are the nonstructural proteins functioning in intracellular virus replication (Fig. 1B).

In infected cells, the genomic RNA is translated, and processing of the polyprotein begins with translocation of the