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RNA Virus Replicon Vaccines

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INTRODUCTION

Expression systems based on single-stranded RNA viruses offer exciting prospects for their application as vaccines. The development of cDNA-based genetic systems for a substantial number of these viruses has made possible the directed manipulation of RNA virus genomes, the discovery of substantial genomic plasticity in these virus systems, and their reconfiguration as vaccine vectors. Pioneering work with infectious clones of poliovirus (1), positive strand RNA plant viruses (2,3), and rabies virus (RV) (4) was key in guiding subsequent development of analogous reverse genetic systems for other RNA viruses and vaccine vectors derived from them.

Single-stranded RNA virus vectors can be divided conveniently into those based on positive or negative stranded genomes. Positive stranded genomes have the same sequence as mRNA, and their genomes are infectious when introduced alone into a cell by any one of a number of transfection methods. For genetic manipulation of a positive strand RNA genome, cDNA clones of the viral RNA are linearized downstream of the viral sequences, and in vitro transcription results in the production of positive strand RNA replicas of the viral genome which also are infectious.

Negative stranded RNA genomes carry their genetic information in the complementary sense relative to mRNA. Therefore, successful virus propagation requires that the viral replicase/transcriptase enzymes required in the earliest stages of intracellular replication be introduced into the cell along with the genome. In systems designed for reverse genetics of negative stranded viruses, in vitro transcripts from cDNA clones (usually transcripts that are the positive sense complement of the genome) are introduced into cells where the replicase proteins are being expressed from a second expression system (often the vaccinia T7 system).

With either positive or negative sense RNA genomes, the systems used to regenerate infectious viruses from cDNA clones have also been employed to add immunizing genes from target pathogens to the viral genomes. This results in a fully infectious and propagation competent virus vector, which expresses an exogenous immunogen in addition to a full complement of viral proteins in the successive cells it infects (Fig. 1A.1). A second iteration on this theme is the substitution of an immunogen gene for one or more of the structural protein genes of the vector virus. The resulting RNA genome is self-replicating inside an appropriate cell, thus the term ''replicon.'' The replicon expresses the immunizing gene and can be packaged into ''replicon particles'' when the structural proteins are produced *in trans* within the same cell (Fig. 1B). When inoculated into an animal or human, the replicon particles target to those cells normally infected first by the complete virus, the replicon genome expresses the immunizing gene, but the infection cannot be propagated to additional cells due to the absence of the structural protein genes from the replicon genome. Alternatively, a positive sense replicon can be transcribed, or launched, from a DNA vaccine in vivo. Thus, RNA replicons delivered as replicon particles or launched from DNA constructs can provide safe and effective immunization against a variety of pathogens.

Table 1 provides a list of RNA virus vectors discussed in this review.

VACCINE VECTORS DERIVED FROM POSITIVE STRAND RNA VIRUSES

Propagation Competent Vectors

Propagation competent vectors express an immunizing gene in the context of a complete virus genome. For instance, a second copy of an alphavirus subgenomic mRNA promoter can be inserted into the genome for expression of an immunizing transgene (5–9).

Coronaviruses express their genes from a 3′ co-terminal nested set of mRNAs. An exogenous immunizing gene may be inserted between native coronavirus genes, and the gene will be expressed if the *cis*-acting intergenic region signals, necessary for transcription of mRNA, are included at its 5′ end (10).

In picornavirus propagation competent vectors, short, foreign epitope sequences have been substituted for external loops on the poliovirus capsid structure (11), or an immunizing gene has been placed either upstream of the major capsid genes or at the P1/P2 junction, maintaining the genomic open reading frame (ORF; 12–14). Crotty et al. (15,16) overcame the inherently limited insertion size in such vectors by expressing a library of relatively short overlapping coding sequences from genes many times larger than the insertion maximum.

Flaviviruses have been modified in a manner analogous to picornaviruses (17,18). In addition, the 17D yellow fever vaccine, as well as attenuated dengue fever virus subtypes, have served as a backbone for flavivirus chimeras in which the prM and E genes of a flavivirus to be vaccinated against are exchanged for the same genes in one of the vaccine virus backbones (19–21) (Fig. 1A.2). The goal is to take advantage of the attenuating mutations embedded in the vaccine backbone to create alternative live virus vaccines for the agents supplying the exogenous prM and E genes. Human clinical trials of this concept are underway (Japanese encephalitis) (22–24).