Table 1 List of RNA Virus Vectors

Positive strand RNA virus vectors	Negative strand RNA virus vectors
Propagation-competent	*Propagation-competent*
Alphavirus	Rhabdovirus
Sindbis virus, VEE virus, Semliki forest virus	VSV, RV
Coronavirus	Paramyxovirus
TGEV, mouse hepatitis virus, SARS coronavirus	Human or bovine parainfluenza virus 3
Picornavirus	Human RSV
Poliovirus	Sendai virus, Newcastle disease virus
	Measles virus, simian virus 5
	Influenza virus
Flavivirus	
Yellow fever virus	
Chimeric virus	*Chimeric virus*
Flavivirus	Paramyxovirus
Yellow fever virus, dengue fever virus	Bovine RSV, human or bovine parainfluenza virus 3
Nonpropagating replicons	*Nonpropagating replicons*
Alphavirus	Rhabdovirus
Sindbis virus, Semliki forest virus, VEE	VSV, RV
Coronavirus	Influenza virus
TGEV	
Togavirus	
Rubella virus	
Picornavirus	
Poliovirus, rhinovirus, mengo virus	
Flavivirus	
Kunjin virus	

Abbreviations: RV, rabies virus; RSV, respiratory syncytial virus; TGEV, transmissible gastroenteritis virus; VEE, Venezuelan equine encephalitis; VSV, vesicular stomatitis virus.

replicon systems and replaced by the immunizing gene. Thus, the gene of interest is expressed in replicon-infected cells to levels approaching 20% of the total cell protein. In the SFV and SIN systems, high level expression depends on the presence of a translational enhancer sequence extending from the subgenomic mRNA start site approximately 200 nucleotides into the capsid gene (38,39). Although the cells are eventually killed as a result of replicon infection, the absence of the structural protein genes prolongs the period of maximal expression (40). Moreover, mutant alphavirus replicons have been selected, which are capable of persistent infection and expression (41–44).

Packaging of alphavirus replicon genomes into replicon particles has been accomplished either by co-electroporation of replicon RNA and helper structural protein genes transcribed in vitro (32–35) or by the establishment of stable cell lines constitutively expressing transcripts for the structural proteins (45). In the packaging cell lines, the helper sequences are under the control of the alphavirus subgenomic RNA promoter, so that although they are constitutively transcribed from the cellular DNA as part of a larger mRNA, they are not transcribed from the subgenomic promoter or translated until introduction of the replicon RNA, either by electroporation or by infection with previously packaged replicon particles. Complementation occurs between the replicase functions encoded by the replicon RNA and the structural proteins supplied by the helper RNAs. Replicon particles are assembled that contain only the replicon RNA due to the absence of a *cis*-acting packaging signal in the helper RNAs.

Alphavirus RNAs are capable of low-level recombination (46), and alphavirus particles can copackage multiple RNAs (33). In the context of alphavirus packaging systems, either can result in the production of propagation competent genomes. Expressing the capsid and glycoprotein genes from separate helper RNAs significantly reduces the generation of propagation competent virions contaminating replicon particle preparations (35,45,47).

Replicon particles provide an efficient system for delivery of the replicon genome into cells in vivo. The effectiveness of the VEE replicon system may be attributable in part to the ability of VEE replicon particles to target and replicate within dendritic cells in lymph nodes of mice (48) and primates (West et al., unpublished data). Human monocyte-derived dendritic cells (DCs) also are targets for VEE replicon particles ex vivo, in which they induce maturation and active presentation of antigen to T cells (49,50). SIN variants, selected from laboratory-adapted strains for increased ability to target dendritic cells, may improve the ability of SIN replicon particles to induce immune responses (51). However, other studies suggest that wild-type SIN itself targets efficiently to dendritic cells in vivo (52). SFV replicon particles also appear to target lymphoid tissue (53), but do not infect professional antigen-presenting cells (54). Perri et al. (55) utilized a chimeric alphavirus system, combining SIN glycoproteins for targeting to cells in vivo and the VEE replicon genome to reduce sensitivity to interferon for better expression.

Replicon RNAs derived from alphaviruses also have been delivered into animals directly (56–60), although degradation of the RNA prior to entry into cells may limit this approach. Alternatively, cDNAs driven by eukaryotic promoters have been used to express self-replicating replicon RNAs (61–66). In the case of cDNA delivery, the efficiency of transcription in and exit from the nucleus may limit effectiveness in a vaccine context (67).

Alphavirus replicon particle vaccines have been tested in a variety of animal models of disease. Humoral, cellular and mucosal immunity have been demonstrated in mice, and in primates there is clear induction of both antibodies and cytotoxic T cells. Induction of protective levels of immunity and/or protection against challenge has been demonstrated with VEE replicon particle vaccines in rodent models of laboratory-adapted influenza (35); a human pathogenic Hong Kong origin H5N1 virus (68); Lassa fever, Ebola, Marburg, Anthrax, and botulinum toxin (69–74); staph enterotoxin (75); Norwalk virus (76,77); Lyme disease (78); gonococcus (79,80); SARS (81); orthopoxviruses (82); dengue fever (83); cytomegalovirus (84,85); respiratory syncytial virus (RSV) (86,87); and human metapneumovirus (88). Protection with VEE replicon particles also has been reported for equine arteritis virus in horses (89,90). In primates, partial protection against simian immunodeficiency virus (SIV) challenge has been demonstrated (91–94), and complete protection of primates against a high dose challenge of Marburg virus was achieved (95). Immunization with VEE replicon particles expressing the E7 protein from human papilloma virus 16 completely protected mice against tumor establishment in a murine model and led to eradication of established tumors in 67% of the animals (96,97). Homologous dendritic cells infected with VEE replicon particles (VRP) expressing her2/neu were effective in both prophylactic and therapeutic settings in wild-type mice but not in transgenic mice harboring