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Review Article

r-DNA VACCINES AND DRUGS FOR HUMAN AND ANIMALS

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ABSTRACT

Recombinant DNA (r-DNA) vaccines based on replicase gene plasmid vectors are best suited for large scale production of human and animal vaccines because they mount a high level of immune response and a very small amount of DNA is required for immunization. The r-DNA drugs need to be based on conventional plasmid DNA vectors because here we do not want to induce immune response but want to deliver proteins for therapeutic use.

Keywords: r-DNA vaccine, r-DNA drugs, replicase gene, pAlpha vector.

INTRODUCTION

The vaccines currently used are either live attenuated or inactivated vaccines. The live-attenuated vaccines induce humoral as well as cell mediated immune responses but their immunogenicity is somewhat lowered in the process of attenuation. The inactivated vaccines although are capable of inducing humoral as well as cell mediated immune responses, these are not long lasting (Ferraro *et al.*, 2011).

DNA based vaccines

DNA vaccines got the attention with the findings that when plasmid DNA was delivered into skin or muscle, they induced antibody responses to viral and non-viral antigens (Tang *et al.*, 1992; Fynan *et al.,* 1993; Ulmer *et al.*, 1993). DNA vaccines could induce broad immune responses similar to live attenuated viruses without the need for a replicating pathogen. Wang *et al.,* (1998) reported that DNA vaccines could activate CD^{8+} cytotoxic T cells (CTL) in larger animal models. In construction of DNA vaccine, the immunogenic component/gene is cloned in a mammalian expression vector in right orientation. The amount of DNA required to produce immune response is 50-100µg in an animal model. However these immune responses were not as effective as desired and hence different strategies were employed to enhance their immunogenicity including use of immuno-stimulatory (CpG) sequences, dendritic cells (DC), co-stimulatory molecules and cytokine-adjuvants (Leitner *et al.,* 2000). One promising approach is making them 'self-replicating). This can be accomplished by using a gene encoding RNA replicase, a polyprotein derived from positive-strand RNA alphaviruses, such as Sindbis virus (Herweijer *et al.*, 1995; Hariharan *et al.*, 1998; Ahi *et al.*, 2008; Miller *et al.*, 2008; Saxena *et al.*, 2008;

Gupta *et al*., 2009; Kumar *et al*., 2009; Kanojia and Rai, 2016), Semliki forest virus (Liljestrom and Garoff, 1991; Zhou *et al*., 1994; Berglund *et al*., 1998; Zhao *et al*., 2009;) and Venezuelan equine encephalitis virus (Davis *et al*., 1989; Lee *et al*., 2003) with ability of these viruses to produce large amounts of viral proteins in infected cells. Alphavirus vectors have demonstrated high levels of transient heterologous gene expression both *in vitro* and *in vivo*. Replicase-containing vectors are significantly more immunogenic than conventional plasmids, immunising mice at doses as low as 0.1µg nucleic acid injected once intramuscularly. Cells transfected with self-replicating vectors briefly produce large amounts of antigen before undergoing apoptic death. This death is a likely result of requisite double-stranded RNA intermediates which also have been shown to super-activate DC. Thus the enhanced immunogenicity of self-replicating gene vaccines may be a result of the production of pro-inflammatory dsRNA, which mimics an RNA-virus infection of host cells (Leitner *et al*., 2000). Transfected cells express the antigen encoded on the plasmid resulting in an immune response engaging both MHC-1 and MHC-2 pathways allowing for the induction of CD^{8+} and CD^{4+} T cells (Wang *et al*., 1998) whereas antigen present in soluble form, such as recombinant protein generally induces only antibody responses. The Sindbis virus replicase gene has been preferably used in construction of the replicase vector and the sequence of components is: 5' CMV promoter- 5' UTR- non-structural genes- 26S subgenomic promoter-immunogenic gene of interest-3'UTR-polyA signal. All alphavirus vectors take advantage of the extremely efficient RNA replication resulting in some 200,000 RNA copies from each RNA molecule (Lundstrom, 2014). SIN-based DNA vaccines have been developed against rabies (Saxena *et al*., 2008) and in comparison to a conventional rabies DNA vaccine, it induced better humoral and cell mediated immune responses in immunized mice and showed complete protection against challenge with CVS rabies virus. The DNA vaccines developed are summarized in Table1.

Table1. r-DNA vaccines developed for human and animals

PATHOGN	GENE	VECTOR	REFERENCES
Hepatitis B	sAg	Sin	Driver *et al*., 1995
Hepatitis C	cAg	DNA	Vidalin *et al*., 2000
HIV-1	Env	SFV	Brand *et al.,*1998
HSV-1	gpB	Sin	Schlesinger *et al.,*1999; Hariharan *et al.,*1998
Influenza	HA	SFV/VEE	Malone *et al.,*1997; Schultz-Cherry *et al.,*2000; Bosworth *et al.,* 2010
Measles	HA	Sin	Pasetti *et al.,*2009; Pan *et al.,*2010

Rabies	G	pTargeT, pSG5, pAlpha	Rai and Yadav, 2001; Rai *et al.,*2002; Rai *et al*., 2005; Ahi *et al.,*2008; Saxena *et al.,*2008; Saxena *et al*.,2009; Gupta *et al.,*2009; Kaur *et al.,*2009; Gangwar *et al.*,2010.
RSV	F,G	SFV/DNA	Fleeton et al,2001; Cheng *et al*, 2002
Distemper	H	pAlpha	Kumar *et al*, 2009
Parvovirus	VP2	pTargeT, pAlpha	Gupta *et al.,*2005, Dahiya *et al.,*2012
Canine hepatitis	Hexon	pTargeT	Salunkhe *et al.,*2008a; Salunkhe *et al.,*2008b
CSFV	E2	pVAX1	Singh *et al.,*2009
IBD	VP2	pTargeT, pAlpha	Chauhan *et al.,*2005;Kumar *et al.*,2008; Kumar *et al*., 2009;
FAV-4	Hexon	pAlpha	Rai *et al.,*2005; Sandey *et al.,*2008
NDV	F, HN		Patel *et al* 2008; Rajawat *et al.,*2008
Malaria	CS	Sin	Tsuji *et al.,*1998; Le *et al.,*2000
B. anthracis	PA	Sin	Thomas *et al.,*2009
M. tuberculosis	Ag85A	Sin	Kirman *et al.,*2003
Cervical cancer	HPV E6-E7	SFV	Daemen *et al.,*2002; Daemen *et al.,* 2004; Cheng *et al.,*2006; Velders *et al.,*2001; Cheng *et al.,* 2002

r-DNA based drugs

Based on the principle of DNA vaccine, we can produce drugs based on this technology but the aim here is not to drive immune response but to produce the therapeutic protein in the host which in turn produces the therapeutic effect. So in this situation, we should use a simple mammalian expression vector which produces the protein which in turn produces therapeutic effect. The plasmid vectors encoding gene of streptokinase, human erythropoietin, human IL-2, human IL-4, human IFN gamma, human IL-18 have been developed which can be used as therapeutic agent. The cloning of VP3 gene of chicken infectious anaemia virus has paved the way for an effective anti-tumour therapeutic vaccine.

Table 2. r-DNA based drugs developed for human

NAME OF DRUG	VECTOR	REFERENCES
Streptokinase	pTargeT	Gangwar *et al.,*2010
Human erythropoietin	pTargeT	Gangwar *et al.,*2009
HumanIL-2	pTargeT	Saxena *et al.,* 2007; Gangwar *et al.*, 2008
Human IL-4	pTargeT	Gangwar *et al.,*2008
Human IFN $^{\gamma}$	pTargeT	Gangwar *et al.*,2008
Apoptin	pTargeT	Thakuria *et al*., 2008
Human IL-18	pTargeT	Gangwar *et al.*, 2008

Lundstrom (2014) has reviewed the relevance and significance of DNA vaccines. Rai *et al* (2009) reported the standardisation of silica gel technology for large scale isolation and purification of plasmid DNA for vaccine/drug use which is efficient and economic.

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