REVIEW ARTICLE

DNA vaccines and their applications in veterinary practice: current perspectives

K. Dhama · Mahesh Mahendran · P. K. Gupta · A. Rai

Abstract Inoculation of plasmid DNA, encoding an immunogenic protein gene of an infectious agent, stands out as a novel approach for developing new generation vaccines for prevention of infectious diseases of animals. The potential of DNA vaccines to act in presence of maternal antibodies, its stability and cost effectiveness and the non-requirement of cold chain have heightened the prospects. Even though great strides have been made in nucleic acid vaccination, still there are many areas that need further research for its wholesome practical implementation. Major areas of concern are vaccine delivery, designing of suitable vectors and cytotoxic T cell responses. Also, the induction of immune responses by DNA vaccines is inconclusive due to the lack of knowledge regarding the concentration of the protein expressed *in vivo*. Alternative delivery systems having higher transfection efficiency and the use of cytokines, as immunomodulators, needs to be further explored. Recently, efforts are being made to modulate and prolong the active life of dendritic cells, in order to make antigen presentation a more efficacious one. For combating diseases like acquired immunodeficiency syndrome (AIDS), influenza, malaria and tuberculosis in humans; and foot and mouth disease, Aujesky's disease, swine fever, rabies, canine distemper and brucellosis in animals, DNA vaccine clinical trials are underway. This review highlights the salient features of DNA vaccines, and measures to enhance their efficacy so as to devise an effective and novel vaccination strategy against animal diseases.

Keywords Animal diseases · Cytokines · DNA vaccines · Recombinant vaccines · Vaccination · Veterinary vaccines

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Abbreviations

APC	antigen presenting cell
CpG	cytosine guanosine motifs
CTL	cytotoxic T lymphocytes
CD	cluster of differentiation or canine distemper
CMI	cell mediated immunity
CMV	cytomegalovirus
DNA	deoxyribonucleic acid
GM-CSF	granulocyte monocyte colony stimulating factor
IM	intramuscular
IL	interleukins
MHC	major histocompatibility antigen
OMP	outer membrane protein
ORF	open reading frame

Introduction

The property of naked DNA to get transfected to mammalian cells *in vivo* was first reported by Ito (1960); and three decades later, the concept of DNA vaccine was evolved by Wolff et al. (1990), when they administered a recombinant bacterial plasmid DNA to obtain the expression of β-galactosidase gene in mice. This paved way for the development of nucleic acid based vaccination, an effective way for the *in vivo* expression of desired protein to initiate immune response (Oshop et al. 2002; Liu 2003). The application of DNA immunization as a new generation vaccine has been well studied since its invention, and a variety of such vaccines have undergone clinical trials, in veterinary practice (Dunham 2002; Oshop et al. 2002; Babiuk et al. 2003; Babiuk et al. 2007). The DNA vaccines elicit desired immune responses viz. cell mediated immunity (CMI) and humoral immune response (HIR); and it is much easier for their manipulation using recombinant DNA techniques and production in bacteria using fed-batch fermentation (Liu 2003; Liu et al. 2006). As an effective vaccine, plasmid DNA have a gene encoding a protective antigen of a pathogen, which when injected into host, is transcribed and translated, to induce a specific immune response. The DNA vaccines, described as genetic immunization to elicit a protective immune response, have been further improved by exploiting various gene delivery methods, cytokine adjuvants and prime-boost (DNA vaccine priming and recombinant protein boosting) approaches (Sharma and Khuller 2001; Jiang et al. 2007).

DNA vaccines have several advantages, which include simplicity of manufacture, biological stability, cost effectiveness and safety, ease of transport in lyophilized form and the ability to act in presence of maternal immunity. Besides, different genes can be combined simultaneously, making it possible to develop multivalent vaccines. The demerits of DNA vaccines, of theoretical levels and not yet proven are; integration into host genome, activation of proto-oncogenes, inactivation of tumor suppressor genes and the possibility of generating anti-nuclear antibodies (Sharma and Khuller 2001; Dunham 2002). However, as the merits of DNA vaccines outnumber the hypothesized demerits, presently they have moved towards second stage clinical trials with promising results, for human diseases like acquired immunodeficiency syndrome (AIDS), herpes infections, rabies, Ebola, tuberculosis, malaria, and Leishmaniosis. However, a commercial product has not reached the market yet, due to the safety concerns raised by the international regulatory organizations. Regarding veterinary practice, the last few years have seen numerous trials of DNA

vaccines against various animal diseases like foot and mouth disease (FMD) and herpes virus infection in cattle, Aujeszky's disease and classical swine fever in swine, rabies and canine distemper in canines, and avian influenza, infectious bronchitis, infectious bursal disease and coccidiosis in birds (Oshop et al. 2002; Dunham 2002; Ding et al. 2005; Gupta et al. 2006; Patial et al. 2007). One of the distinct advantages of the DNA vaccines is the possibility of differentiating infected from the vaccinated animals (DIVA), for effective disease eradication programs. The utility of 'marker' DNA vaccines has been reported for diseases like FMD and avian influenza (Lee et al. 2004; Grubman 2005). Even though, DNA vaccines has ushered a new era in veterinary vaccinology, the potential of the vaccine to develop higher levels of immune response, has to be further improved. Keeping in view of the appealing features, and ease of generation of DNA vaccines, in the present review, authors have meticulously portrayed the salient features of DNA vaccines, ways to improve its efficacy, and their potential applications in veterinary practice.

Salient features of DNA vaccines and strategies to improve vaccine efficacy

DNA vaccines, generated using plasmids, include a gene encoding target antigen under the transcriptional control of an effective viral/eukaryotic promoter, along with a poly-adenylation signal sequence (poly-A) and a bacterial origin of replication (Fig. 1) (Gurunathan et al. 2000). The commonly used promoter has been derived from cytomegalovirus (CMV). The poly-A provides stability and effective translation; and the antibiotic resistance gene facilitates selection of bacteria (Gurunathan et al. 2000; Sharma and Khuller 2001; Liu 2003; Brandsma 2006). For complete optimization, the plasmid should have Kozak sequence (GCCA/GCC) upstream of initiator codon, and an enhancer, down stream of the poly-A signal (Gurunathan et al. 2000). As a vaccine, the amount of plasmid DNA required for IM administration is 10-100 μg in mice, 100-300 μg in small animals and 0.5-2.5 mg in large animals and humans; but, while using 'gene gun', only one hundredth of this amount is required (Dunham 2002). If properly optimized, the recombinant plasmids are capable of expressing the desired antigen *in vivo* (Dunham 2002; Babiuk et al. 2003; Babiuk et al. 2007). But, to elicit an effective immune response, the protein should undergo post-translational modification and retain their tertiary structure. After *in vivo* generation, the antigenic peptides are processed and presented by professional antigen presenting cells (APCs), like dendritic cells, by getting primed through direct transfection or by obtaining proteins from myocytes via cross presentation (Fig. 2) (Fu et al. 1997), to further allow presentation via both MHC I and II, to induce cellular and humoral

Fig. 1 Diagramatic representation showing essential features of a DNA vaccine construct. Transcription unit comprises of a promoter, desired immunogenic or protective gene and a polyadenylation signal sequence. A bacterial origin of replication (ori) and an antibiotic resistance gene are also incorporated in the vector back bone to permit growth and selection of the plasmid in bacteria
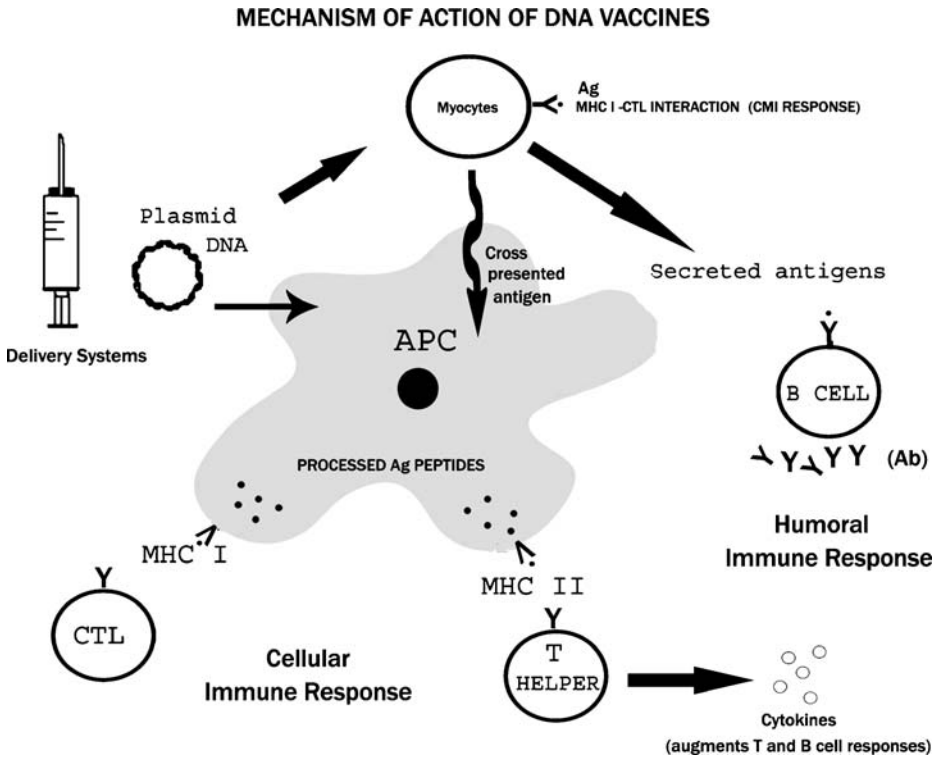
MECHANISM OF ACTION OF DNA VACCINES
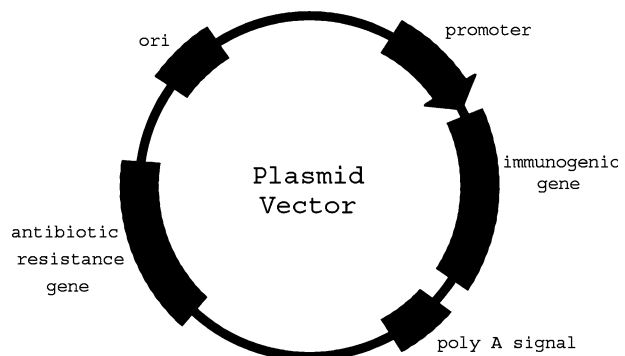
Fig. 2 A pictorial representation of generation of antigen-specific cellular and humoral responses by DNA vacccines. Professional APCs like dendritic cells and macrophages receives the secreted antigens (cross presentation) from transfected somatic cells or directly get transfected. They process and present the antigenic peptides to major histocompatibility complex (MHC) Class II molecules for helper T cells, which releases a variety of cytokines to augment activation of various cells of immune system. Activation of cytotoxic T lymphocytes occurs by degraded antigenic peptides that are associated with MHC Class I molecules. These two mechanisms help in the generation of cellular immune responses. For humoral or antibody responses, B lymphocytes recognize and respond to antigens that are present extracellularly, or as secreted antigens

immunity. However, in some cases, DNA vaccines have failed to produce measurable antibodies even when the host got protected, suggesting a major role for cellular immune responses (Seo et al. 1997; Kodihalli et al. 2000). In addition, the potential of DNA vaccines to act in presence of maternal antibodies is remarkable, due to the ability to provide a durable source of the antigen by dendritic cells. Similarly, when administered with cationic liposomes, they enhance mucosal immunity, to protect the host from respiratory or enteric infections. Besides, researchers have suggested the immunogenic properties of the plasmid itself, by virtue of having CpG motifs, functioning as adjuvant and up-regulator of natural killer (NK) cells (Krieg 2002).

For improving DNA vaccines, efforts have been made to enhance their efficacy, through efficient vectors, and vaccine delivery systems that combines delivery route and formulation. In animals, DNA vaccines are found less effective due to host impedance, improper transfection and low level of expression. But, various delivery formulations and route could enhance the immune response. Delivery methods like suppositories (Loehr et al. 2001), needle free injector system (van Rooij et al. 1998), mucosal delivery (Barnes et al. 2000) and topical application (Oshop et al. 2002), have been found useful. Vaccination

using gene gun, help in direct transfection of dendritic cells, favoring effective antigen presentation (Dunham 2002; Ulmer et al. 2006). Also, by using cationic lipid complexes and cytokine adjuvants, the efficacy could be significantly improved (Min et al. 2001; Stevenson 2004; Manoj et al. 2004a; Dhama et al. 2007). However, the major lacuna that still persists is the lack of simultaneous generation of CMI and HIR. Hence, researchers have evolved various options to improvise DNA vaccines for increasing CMI and HIR together. Addition of a eukaryotic secretory signal sequence have been found to improve the activity of cytotoxic and helper T cells. Also, linking of ubiquitin molecule to enhance the proteasome-based degradation, and the use of toll like receptor (TLR) adapter molecule (MyD88) along with the desired gene, may elicit a strong CMI and HIR. Further, the use of L-selectin or cytotoxic T lymphocyte antigen (CTLA-4) ligands help better antigen targeting to the immune cells (Chaplin et al. 1999). Similarly, targeting of the dendritic cells by heat shock proteins (HSP); microparticulate formulations, and complexing with non-ionic block co-polymers, polycations or cochleates, may also increase vaccine efficacy (Manoj et al. 2004a). Targeted intra-cellular delivery of plasmid DNA can be further achieved using intracellular bacteria and viruses (Liu 2003; Daudel et al. 2007). Recently, prime-boost regimens and ways to increase the functional life of dendritic cells have been explored (Ulmer et al. 2006; Tsen et al. 2007). Also, 'electroporation', few days prior to vaccination, has been found to force plasmid into cells or promoting influx of inflammatory cells like macrophages in myocytes (Babiuk et al. 2007; Peng et al. 2007). Further, nanoparticle-mediated plasmid delivery has been suggested for augmenting the immune responses (Jiang et al. 2007).

Applications of nucleic acid vaccines in veterinary practice

Veterinary vaccinology is a rapidly developing field and currently, vaccines are not only used for the prevention of diseases in animals, but also to help solve public health crisis, by effectively checking emerging or re-emerging pathogens of zoonotic significance. Advancement in science and technology, together with improved knowledge in immunology, microbiology and recombinant technology has played pivotal roles in introducing novel ideas in vaccinology (Babiuk et al. 2003; Liu et al. 2006). Shams (2005), has pointed out that subunit vaccines, DNA vaccines and vectored vaccines are rapidly gaining acceptance as new generation animal vaccines. The last few years have seen the development of nucleic acid vaccines against many diseases like classical swine fever, Aujeszky's disease, rabies, foot and mouth disease, brucellosis, bovine tuberculosis, equine herpes infections, avian infectious bronchitis, avian influenza, infectious bursal disease, chlamydiosis and avian coccidiosis (Table 1). DNA vaccines have promoted a revolution in the concept of vaccination, which has been previously dominated by inactivated or live vaccines. Among many advantages, DNA vaccines also provide DIVA strategy, and hence vaccine-induced herd/flock immunity can be differentiated for effective sero-surveillance (Lee et al. 2004). Hence, the latest research development in the field of nucleic acid-based vaccination strategy for preventing various bacterial and viral diseases of domestic animals as well as poultry has been discussed further in detail.

Bovines Genetic immunization approach against bacterial diseases of bovines offers attractive possibilities for rapid and effective vaccine development. Against brucellosis, an IM administered L7/L12 gene has been reported to result in intracellular expression of the immunodominant L7/L12 protein (Kurar and Splitter 1997). *Brucella abortus*

Table 1 DNA vaccines for infectious diseases of Veterinary importance

Disease	Host	Etiological agent	Protective antigen gene	References
Bacterial Diseases				
Brucellosis	Cattle	*B. abortus*	L7/L12; SOD	Kurar and Splitter 1997; Rivers et al. 2006
Tuberculosis	Cattle	*M. bovis*	MPB83; Ag85B	Chambers et al. 2000; Teixeira et al. 2006
Mastitis	Cattle	*S. aureus*	FnBP; ClfA	Nour El-Din et al. 2006
Anthrax	Sheep	*B. anthracis*	PA83	Hahn et al. 2006
Johne's Disease	Ovines, Caprines	*M. avium paratuberculosis*	HSP-65	Sechi et al. 2006
Brucellosis	Caprines	*B. melitensis*	OMP-31	Gupta et al. 2007
Leptospirosis	Canines	*L. canicola*	flaB2	Dai et al. 2003
Broncho-pneumonia	Equines	*Rhodococcus equi*	VapA	Vanniasinkam et al. 2005
Colibacillosis	Poultry	*E. coli*	K88	Cho et al. 2004
Chlamydiosis	Poultry	*C. psittaci*	MOMP	Vanrompay et al. 2001
Viral Diseases				
Bovine leukemia	Cattle	Retrovirus	gp51; gp30	Brillowska et al. 1999
Infectious bovine rhinotracheitis	Cattle	Bovine herpes virus	gC; gD	Gupta et al. 2001; Manoj et al. 2004b
Bovine viral diarrhea	Cattle	Pestivirus	E2	Nobiron et al. 2003
Foot and mouth disease	Cattle	Picornavirus	VP1	Dong et al. 2005
Swine fever	Swine	Pestivirus	E2	Wienhold et al. 2005
Pseudorabies	Swine	Herpes virus	gB; gC; gD	Gerdts et al. 1999
Parvoviral infections	Canines	Parvovirus	VP1; VP2	Jiang et al. 1998; Gupta et al. 2005a
Rabies	Canines	Rhabdovirus	gp gene	Rai et al. 2005
Canine distemper	Canines	Morbilivirus	HA; F	Sixt et al. 1998
Equine influenza	Equines	Influenza virus	HA	Lunn et al. 1999
Equine herpes infection	Equine	Herpes virus	gB; gC; gD	Minke et al. 2006
Avian influenza	Poultry	Influenza virus	HA	Kodihalli et al. 2000
Newcastle disease	Poultry	Avian paramyxovirus	HN; F	Loke et al. 2005
Infectious bronchitis	Poultry	Coronavirus	N; S1	Seo et al. 1997
Infectious bursal disease	Poultry	Avibirnavirus	VP2	Li et al. 2006
Chicken infectious anemia	Poultry	Gyrovirus	VP1 and VP2	Senthil Kumar et al. 2004
Other diseases				
Anaplasmosis	Bovines	*A. marginale*	MSP1b	de Andrade et al. 2004
Cryptosporidiosis	Caprines	*C. parvum*	15 kDa	Sagodira et al. 1999
Schistosomiasis	Caprines	*S. japonicum*	Sj28GST; Sj23	Shi et al. 2001
Babesiosis	Canines	*B. gibsoni*	p50	Fukumoto et al. 2007
Coccidiosis	Poultry	*E. tenella, E. acervulina*	3-1E; EtMIC2	Ding et al. 2005

glyceraldehyde-3-phosphate-dehydrogenase (GDPH), a T and B cell reactive protein, which could induce partial protection when co-administered with IL-12; and the copper-zinc super oxide dismutase (SOD) antigen of *B. abortus* has also been utilized for the generation of an effective DNA vaccine (Rivers et al. 2006). Against mycobacterial infections in cattle, Huygen (2003) has explained the utility and feasibility of the DNA vaccine approaches. DNA vaccine based on *Myobacterium bovis* protein MPB-83 when tested in mice has shown to elicit protective immune responses (Chambers et al. 2000). The use of co-stimulatory molecule CD 154 along with ESAT-6 gene has been suggested to enhance the immunogenic properties of mycobacterial DNA vaccines. Further, *M. bovis* Ag85B gene has been found suitable while incorporating in plasmid vector due to its ability to induce a Th1 type of immune response (Teixeira et al. 2006). Aside to this, recently, DNA vaccines used in combination with Bacillus Calmette Guerin (BCG) elicited superior protection during challenge studies (Cai et al. 2006). For the control of staphylococcal mastitis in bovines, clumping factor A (ClfA) or fibronectin binding protein (FnBP) genes of *S. aureus* are considered while developing DNA vaccines. However, a DNA vaccine comprising of ClfA gene alone, could induce sufficient protection against *S. aureus* infection (Nour El-Din et al. 2006; Dhama et al. 2007).

Against viral diseases of bovines, the earliest reports suggest the use of a gene encoding the VP4 protein of bovine rotavirus (BRV), found effective in stimulating a Th1-like immune response (Suradhat et al. 1997). Later, Brillowska et al. (1999) reported the generation of an effective cellular immune response with the plasmid encoding envelope glycoprotein gp51 and transmembrane glycoprotein gp30 of the bovine leukemia virus (BLV). Also, DNA vaccine encoding the fusion (F) gene of bovine respiratory syncytial virus (BRSV) has been found to induce protection against the infection in calves. Besides, several workers have developed successful DNA vaccine strategies against bovine herpes virus-1 (BHV-1) infection (Loehr et al. 2001; Gupta et al. 2001; Castrucci et al. 2005). Gupta et al. (2001) reported that DNA immunization with gC gene of BHV-1 could induce neutralizing antibody and lympho-proliferative responses in bovines. Also, BHV-1 gB gene along with IL-12 has been suggested to enhance the CTL responses. Further, suppositories containing plasmid coding for gD gene of BHV-1 induced mucosal immunity (Loehr et al. 2001); and bovine CD 154 co-stimulatory molecule linked to gD gene enhanced the immune responses (Manoj et al. 2004b). It has also been suggested that gD gene confers higher protection when compared to gC gene. Further, the intercellular trafficking ability of BHV-1 VP22 protein has been utilized to improve the efficacy of a DNA vaccine encoding gD gene (Zheng et al. 2005). Nucleic acid vaccine that could protect the cattle against bovine viral diarrhea virus (BVDV) infection has also been developed. Plasmid DNA, expressing the BVDV type 1 glycoprotein E2, induced virus-specific neutralizing antibodies (Nobiron et al. 2003). Non-structural protein NS3 could also be used for inducing humoral immunity against BVD. Further, Liang et al. (2006) found that the DNA prime boost regimens were effective for preventing BVD in cattle when compared to the administration of DNA vaccine or protein vaccine alone. VP1 based DNA vaccines are being utilized for developing effective vaccines against foot and mouth disease (FMD) (Dong et al. 2005). Plasmid DNA encoding the FMDV VP1 protein followed by boosting with a VP1 peptide conjugate resulted in production of high titers of neutralizing antibodies, suggesting that prime-boost strategy could be a key factor for the success of DNA vaccine against FMD (Jin et al. 2005). Also, the use of IL-1 along with VP1 gene may provide enhanced immune response (Shao et al. 2005). Recently, a microparticulate based DNA vaccine has been developed that codes for the T and B cell epitopes of VP1 of the FMDV (Wang et al. 2006).

DNA vaccines are also being reported against some rickettsial diseases and ecto-parasites of bovines. Vaccine containing the gene of a major surface protein, MSP1b, of *Anaplasma marginale*, offered partial protection against challenge infection (de Andrade et al. 2004). DNA constructs involving ORF of genes, cpg1 and GroEL and GroES of *Ehrlichia ruminantium* could partially protect cattle against "heart water" disease. The potential of DNA immunization with plasmid encoding antigen Bm86 to induce humoral and cellular immune responses against the tick, *Boophilus microplus*, has also been studied (Ruiz et al. 2007).

Ovines and caprines Nucleic acid vaccines have been developed that could confer protection to the common bacterial diseases of sheep and goats. DNA vaccination with genetically detoxified phospholipase D of *Corynebacterium pseudotuberculosis*, linked with CTLA-4, protected sheep against caseous lymphadenitis (Chaplin et al. 1999). In case of anthrax in sheep, the protective antigen (PA83) gene of *Bacillus anthracis* has been employed for developing highly promising DNA vaccine (Hahn et al. 2006). Para-tuberculosis or Johne's disease, caused by *Mycobacterium avium subsp. paratuberculosis,* has been successfully controlled by using plasmids coding mycobacterial heat shock protein antigen (HSP-65) (Sechi et al. 2006). Against brucellosis, administering DNA vaccine that encode *Brucella melitensis* outer membrane proteins (OMP), invasion protein B (ialB), periplasmic protein (bp26) and trigger factor (tF) have been found to induce significant immune responses, which could pave way for the effective control of brucellosis in goats (Yang et al. 2005; Gupta et al. 2007).

For preventing viral diseases of the small ruminants, DNA vaccines have been developed that could protect against diseases like caprine arthritis-encephalitis (CAE), foot and mouth disease (FMD), Visna-Maedi and Rift valley fever. A plasmid expressing CAE viral envelope gene with prime-boost vaccination strategy has shown to induce protective responses in goats (Cheevers et al. 2003). If using gene gun-based mucosal DNA immunization against Visna-Maedi in sheep, the plasmid expressing the envelope gene of the virus in combination with IFNγ gene, is expected to give significant protection and also could restrict virus replication. For preventing FMD infection, Niborski et al. (2006) reported the development of a VP1-based nucleic acid vaccine and found that it was more effective when used along with poly lactide co-galactide (PLG) formulation. Against rift valley fever, the viral glycoprotein gene of Rift valley fever virus (RVFV) when used as DNA vaccine have been found to induce neutralizing antibodies during experimental studies. Recently, Babiuk et al. (2007) reported that a single DNA vaccination with the hepatitis B virus surface antigen gene (HBsAg), in combination with electroporation, approached the efficacy of the commercial subunit vaccine in maintaining long-term protective serum antibody titers against hepatitis B virus in sheep.

DNA vaccines are also being developed against many ecto- and endo-parasites of small ruminants. Vaccination of sheep with a plasmid for the *Boophilus microplus* antigen Bm86 co-administered with plasmid encoding for ovine granulocyte monocyte-colony stimulating factor (GM-CSF) provided significant levels of protection against *B. microplus* infestation (De Rose et al. 1999). An effective recombinant plasmid, encoding the 15 kDa sporozoite surface protein of *Cryptosporidium parvum* has been developed for goats, which provided protective maternal immunity to offsprings (Sagodira et al. 1999). Also, *Schistosoma japonicum* genes (Sj28GST and Sj23) in DNA vaccine formulation offered partial protection as evidenced by a reduction in parasite counts (Shi et al. 2001). Similarly, a nucleic acid vaccine against tapeworms, using the 45W gene of *Taenia ovis*, a protective membrane bound antigen, showed optimum humoral response (Drew et al. 2000).

Swine Regarding the DNA vaccines developed against bacterial diseases in swine, very few research works have been reported. Against swine enzootic pneumonia (SEP), caused by *Mycoplasma hyopneumoniae*, plasmid DNA coding the heat shock protein gene (P42) should be a suitable candidate as it is capable of inducing both Th1 and Th2 immune responses. Against SEP, the capability of P97 adhesin repeat region of *M. hyopneumoniae* to produce immunogenicity in mice in DNA vaccine formulation has also been described (Chen et al. 2006).

However, considerable research has been directed towards developing DNA vaccines to prevent swine fever, FMD and psuedorabies infection in pigs. Plasmid constructs have been developed that could protect swine against the classical swine fever (CSF), which causes significant losses to the pig industry in many Asian and European countries (Wienhold et al. 2005; Andrew et al. 2006). Immunization of pigs with a plasmid expressing the complete E2 protein of classical swine fever virus (CSFV), conferred protection against viral challenge; and the co-delivery of IL-3, IL-18 and CD 154 further enhanced the protective responses (Wienhold et al. 2005; Andrew et al. 2006; Li et al. 2007). Regarding FMD in swine, DNA vaccines encoding VP1 gene of O, A and C strains of FMD virus showed protection against the disease when administered using 'gene gun' (Benvenisti et al. 2001). Two VP1 epitopes (amino acid residues 141-160 and 200-213) have also been found suitable to elicit both FMDV-specific T cell proliferation and neutralizing antibodies (Wong et al. 2002). Researchers have also developed successful DNA vaccines using prM and envelope (E) genes for Japanese encephalitis virus and nucleoprotein (N) gene for transmissible gastroenteritis virus. For constructing DNA vaccines against psuedorabies (Aujesky's disease), the immunogenic viral protein genes such as gB, gC and gD, are often considered (van Rooij et al. 1998; Gerdts et al. 1999; Dory et al. 2005). Plasmids encoding these glycoproteins, when co-injected with CpG motifs, improved the humoral immune response and provided better clinical protection against lethal psuedorabies infection (Dory et al. 2005). Also, it has been suggested that gB and gD genes primes the immune system efficiently even in the presence of maternal antibodies. The utility of dimethyl-dioctadecyl-ammonium (DDA), as an adjuvant to psuedorabies DNA vaccine, has also been reported. For the prevention of porcine reproductive and respiratory syndrome (PRRS) in swine, DNA immunization strategies could be formulated utilizing the viral ORF 5 region that codes for a major envelope glycoprotein GP5. For endo-parasitic infestations like taeniasis (cysticercosis), recently a DNA vaccine using *Taenia solium* B antigen has been developed (Guo et al. 2007).

Canines During the last couple of decades, immunoprophylactic agents have been developed that have greatly reduced the incidence of infectious diseases of pet animals. In canines, even though live vaccines have been found superior in efficacy, it is expected that new generation vaccines may dominate the market in the near future. Focus has been directed for developing DNA vaccines to eliminate the bacterial diseases like leptospirosis and Lyme disease. Leptospirosis, being a zoonotic disease is being given due attention, and many works has suggested the generation of successful vaccines based on its endoflagellar (flaB2) gene. There exist CpG motifs within the flaB2 gene, which could give the DNA vaccine an additional immunostimulatory property, with out the use of adjuvants (Dai et al. 2003). Also, hemolysis associated protein (HAP1) gene has been found to elicit protection against leptospirosis when encoded in plasmids. Against *Borrelia burgdorferi*, a spirochete causing Lyme's disease, DNA vaccine experiments are under trial stages. The role of outer surface protein genes (OspA and OspC) of *B. burgdorferi* to elicit protective immune responses when administered as DNA vaccines has also been explored.

Among the viral diseases affecting dogs, the important ones are rabies, canine distemper and parvoviral infections, many studies have been conducted to analyze the utility of DNA vaccines. Initially, it was against the deadly rabies, the first nucleic acid vaccine was successfully developed (Xiang et al. 1994). After this, it was Jiang et al. (1998) who reported a plasmid DNA vaccine to protect the canine population from parvovirus infections, utilizing VP1 gene of canine parvovirus (CPV). Later, by using VP2 gene of CPV, a successful DNA vaccine was developed (Gupta et al. 2005a). Now, most of the research has been centered on for developing DNA vaccines against rabies and canine distemper (CD). The advantage of plasmid-based vaccine against rabies is that it is a valuable alternative for the mass production of cheaper rabies vaccine when compared to the cell culture based ones. A DNA vaccine encoding the rabies virus glycoprotein (G) has been found to yield stronger and more durable virus neutralizing antibody titers in dogs, which has been proven beyond doubt by many researchers (Rai et al. 2005; Gupta et al. 2005b); and the role of trans-membrane domain of the rabies virus glycoprotein in assisting humoral immune response has been experimentally demonstrated (Gupta et al. 2006). Further, the utility of a chimeric glycoprotein gene, constructed from different Lyssa viruses, can be used as a multivalent vaccine. Also, some works have suggested the advantage of DNA vaccine administered intra-dermally in ear in inducing long lasting protective titer against rabies. Recently, a bicistronic multivalent DNA vaccine has been developed by Patial et al. (2007), which comprised of rabies virus (G) and parvovirus (VP2) genes for inducing neutralizing antibodies against both viral pathogens. It was Sixt et al. (1998) who first developed a plasmid vaccine against canine distemper (CD) that encoded the hemagglutinin (HA) and fusion protein (F) gene against this highly infectious and lethal disease of pups. Also, plasmids containing the nucleocapsid (N), F and HA genes of a virulent CD virus strain can be developed which could elicit strong humoral and cellular immune responses (Jain et al. 2007). Further, immunization with N protein-based DNA vaccine against CD to elicit serum N-specific IgG responses and thereby generating satisfactory protection should also be considered as a viable option. Similarly, the use of HA and F protein genes in a cationic lipid formulation should work well for protecting the pups from clinical disease even in the presence of high titers of maternally derived antibodies.

DNA vaccines are also being attempted for canines, targeting protozoan diseases (Fukumoto et al. 2007). The gene coding the *Babesia canis* protein p50 has been found to induce protective immunity against canine babesiosis, while the p36/LACK antigen gene has been used against Leishmaniosis. Likewise, plasmid DNA encoding a xenogenic tyrosinase can also function as tumor vaccine for prolonging the survival of dogs in cases of malignant melanoma.

Equines: The increasing international movement of horses and the relaxation of regulations have resulted in an increased incidence of equine infectious diseases. Vaccination, along with management measures has become the primary method for the effective control rhodococcal infections, equine influenza, African horse sickness and herpes infections. The advent of recombinant technology has encouraged the development of new generation vaccines such as live-vectored vaccines and DNA vaccines (Minke et al. 2006). Among bacterial diseases, DNA vaccines have been generated to protect the foals from *Rhodococcus equi*, which causes pyogranulomatous broncho-pneumonia. Nucleic acid vaccine, expressing the virulence associated protein (VapA) gene of *R. equi*, induced an anamnestic response and has been found capable in generating specific IgG antibodies (Vanniasinkam et al. 2005).

Against the equine herpesvirus-1 (EHV-1), a viral pathogen of horses causing respiratory, reproductive and neurological problems, the role of plasmid DNA

encoding the envelope glycoprotein D (gD) to induce humoral response, has been suggested; and the administration of GM-CSF along with such vaccines significantly enhanced virus neutralizing antibody responses to EHV-1 (Minke et al. 2006). For equine influenza (EI), Lunn et al. (1999) recorded complete protection of the experimental ponies during challenge after administering DNA vaccine encoding hemagglutinin (HA) gene of EI virus. Aside to this, administration of IL-6 along with HA gene enhances vaccine efficacy and protection (Olsen 2000). To prevent the West Nile virus infection in horses, the envelope protein genes (prM and E) have been incorporated in DNA vaccine formulation to elicit satisfactory protection (Hall and Khromykh 2004). Similarly, Giese et al. (2002) developed effective DNA vaccines encoding the ORF's (5 and 7) of Equine arteritis virus (EAV). Development of nucleic acid vaccines has also been attempted for African horse sickness (VP2 gene) as well as against Vesicular stomatitis (envelope gene).

Poultry Historically, inactivated whole viruses with various adjuvant systems or live vaccines have been used for the successful prevention of various bacterial and viral diseases of poultry. However, the development of naked DNA immunization as third generation vaccines has been well studied and recently a variety of such vaccines are in clinical trials for their use in poultry (Oshop et al. 2002; Dhama et al. 2006). A plasmid DNA vaccine encoding the enterotoxigenic *Escherichia coli* K88 fimbrial protein elicited satisfactory protection during *E. coli* challenge (Cho et al. 2004). Similarly, plasmids expressing the major outer membrane protein (MOMP) of *Chlamydophila psittaci* serovar A and D strains have been found to induce protective immunity with significant reduction in clinical symptoms (Vanrompay et al. 2001). Also, higher levels of protection against *C. psittaci* could be obtained while administering interferon gamma (IFN-γ) or vitamin D, along with DNA vaccines.

Besides, DNA vaccines have been developed against major viral infections of poultry like avian influenza, utilizing the HA gene of the virus (Kodihalli et al. 2000; Lee et al. 2004). Similarly, a vaccine encoding fusion (F) and haemagglutinin (HN) gene induced higher level of antibodies against Newcastle disease (NDV) in chickens (Loke et al. 2005). For Marek's disease, DNA vaccine containing the clone of virulent serotype-1 MDV has been found useful during challenge infection in birds. Vaccination with a mutated, non-oncogenic v-src gene construct, derived from avian leucosis virus (ALV), induced cytotoxic T-lymphocytes (CTL) to protect birds from tumors. Protective utility of plasmid coded N protein gene of infectious bronchitis virus (IBV) has also been reported for which DNA vaccines expressing the S1 glycoprotein of IBV has been suggested (Seo et al. 1997). Against infectious bursal disease (IBD), VP2 gene or VP2/4/3 poly-protein gene of IBD virus-based DNA vaccination has been found effective in protection (Li et al. 2006). DNA vaccination against duck hepatitis B virus has been shown to reduce viremia with rapid removal of the virus from the blood after the challenge. Against chicken infectious anemia (CIA), for the first time, the simultaneous *in vitro* and *in vivo* expression of viral proteins VP1 and VP2 has been studied for generating protective antibodies against the infection (Senthil Kumar et al. 2004). Also, DNA vaccines have been developed against avian reovirus (σC protein gene), and egg drop syndrome (EDS-76) virus (penton fiber gene fragment), and both these vaccines were found effective during their respective challenge studies. Likewise, against protozoan infections, especially coccidiosis, successful DNA vaccines have been developed, utilizing the 3-1E and EtMIC2 genes in combination with cytokines to provide protection from this economically important infection of birds (Min et al. 2001; Ding et al. 2005).

Future prospects

Vaccination with DNA is one of the most promising novel immunization techniques against pathogens, for which conventional vaccination regimens have been less effective. After about 15 years of experimentation, DNA vaccines, nick named 'immunological silver bullet', have become well established in clinical trials. However, they have yet to proceed past the second phase trials primarily due to the inability to induce more potent immune responses in higher mammals. In small experimental animals, the milder host impedance has permitted the DNA vaccines to induce lasting protective effects in contrast to much tougher host barriers in large animals. Significant efforts have been put forward to identify methods of enhancing the immune response of plasmid DNA to enable its practical implementation. Prime importance has been given to develop vaccines to elicit both humoral and cellular immune responses. Researchers have tried a variety of immune modulators, cytokines and co-stimulatory molecules, in this regard. If the potency is improved, plasmid DNA vaccines, having numerous advantages, can be useful for the active immunization against infectious diseases of animals. Considering the current trends and myriad possibilities, efforts should be targeted towards improving their delivery or to increase their immunogenic potential. Poor cellular uptake and rapid *in vivo* degradation of plasmid DNA has to be taken into account and novel delivery systems has to be developed along with the optimization of the plasmid vector. The major challenge in future is the improvement of the transfection efficiency of the DNA vaccines. Gene gun and electroporation can increase transfection and improve immune responses significantly, but these technologies have not yet advanced to routine use in animals. Another promising approach is the development of microparticles as delivery systems or the non-invasive plasmid DNA immunization. Although the potency of the immune response has been weak while using topical application methods, stratum corneum disrupting agents and novel adjuvants may significantly improve them. Further, the properties of DNA vaccines have to be modulated via using cationic liposomes for promoting mucosal and systemic immunity, simultaneously. The current scenario of incorporating such novel methodologies unveils much promise regarding the development of effective, safe and economically viable nucleic acid vaccines. In this context, one should be optimistic regarding the continual research efforts for global implementation of DNA vaccines as an effective immunological arsenal, which could ably address the threats posed by emerging and highly threatening infectious agents of animals.

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