DNA Pharmaceuticals

Formulation and Delivery in Gene Therapy,
DNA Vaccination and Immunotherapy

Edited by
Martin Schleef

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Preface

The difference between "pharmaceuticals" and "modern" or „innovative pharmaceuticals" like nucleic acids (e.g. plasmids, DNA fragments, RNA, viruses or virus-like-particles) is more or less open to interpretation of those developing these to improve safety, functionality, stability or economic aspects (in production and marketing). However, no doubt exists on the existence of a completely new class of active pharmaceutical ingredients (API) when the use of such genetic material for a preventive or curative application was discovered. On one side the need for new products with respect to patent situation and marketing is eminent and on the other side safety concerns for patient and environment are discussed. Furthermore questions like "why changing to a new type of product if the old one still works" are not rare and need to be addressed on the level of market supply costs (were DNA is not expensive) rather than comparing dose costs for existing pharmaceuticals with those for pre-clinical or phase I and II clinical material.

Earlier (in Schleef: "Plasmids for therapy and vaccination", Wiley-VCH 2001) we presented the vector type and clinical approaches of plasmid vectors. This new book extends those subjects into the next step after design and manufacturing of plasmid DNA pharmaceuticals: The focus is on the route of administration, quality control and regulatory aspects.

After a short overview on DNA vaccination (Chapter 1) and a comprehensive summary of regulatory aspects for this class of pharmaceuticals (Chapter 2), the new aspects of improving functionality (e.g. targeting) and purity (ccc-form of plasmid DNA vs. other topologies and contaminants as well as production technology; Chapter 3) or minimizing the vector system (Chapter 4; further progress is expected shortly) are presented.

A special overview on formulation and delivery is presented with Chapters 5 and 6 is a successful example for large animal veterinary DNA vaccine development.

Chapters 6 to 16 indicate the important (different) ways of introducing the vector to the tissue (and cell compartment) of interest. Due to a recently increased interest in electro gene transfer we decided to have two chapters (Chapters 11 and 12) on this subject included. The use of plasmid based siRNA technology was found to be of interest and an example is presented within Chapter 13.

We are aware of the fact that these 13 chapters only represent a small part of the ongoing development in this highly dynamic field. The economic and social relevance of the innovative class of these pharmaceuticals is clearly visible.

For all those who like to further discuss these aspects I look forward to do so at any time (martin.schleef@plasmidfactor.com). My thank is directed to all authors and co-authors of this book and all others making it possible.

Special thanks go to all volunteers of clinical trials with DNA pharmaceuticals.

Bielefeld, August 2005 *Martin Schleef*

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Abbreviations

$\Delta\Psi_i$	induced cell transmembrane potential
$\Delta\Psi_0$	resting cell transmembrane potential
$\Delta\Psi_t$	threshold cell transmembrane value
AAT	α-1-antitrypsin
AAV	adeno-associated virus
ADA	adenosine deaminase deficiency
AGE	agarose gel electrophoresis
APC	antigen-presenting cell
APIs	active pharmaceutical ingredients
ATA	aurintricarboxylic acid
BÄK	"Bundesärztekammer" (Germany)
BCA	bicinchoninic acid
BMP-4	bone morphogenetic protein 4
CAR	coxsackie and adenovirus receptor
CAT	chloramphenicol acetyl transferase
CBER	Center for Biologics Evaluation and Research (USA)
ccc	covalently closed circular
CCCD	conductively connect charge-coupled device
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane conductance regulator
CGE	capillary gel electrophoresis
CIA	collagen induced arthritis
CMV	cyto megalo virus
COPROG	copolymer-protected gene vector
CpG	CpG dinucleotide
CTL	cytotoxic T lymphocyte
CTLA-4	cytotoxic T lymphocyte antigen 4
DC	dendritic cell
DC-Chol	3 beta (N(N′,N-dimethylaminoethane)carbamoyl) cholesterol
DEAE	diethylaminoethyl-

DH	Department of Health (UK)
DMF	drug master file
DMPE	dimyristoyl phosphatidylethanolamine-
DMRIE	1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethylammonium bromide
DOPE	dioleoylphosphatidylethanolamine
DOTAP	1,2-dioleoyl-3-trimethylammonium-propane
DOTMA	N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride
DPI	dry powder inhaler
EAV	equine arteritis virus
ECT	electrochemotherapy
EGF	epidermal growth factor
EGT	electrogenetherapy
EHD	electrohydrodynamic
ELISA	Enzyme-linked immunosorbent assay
EMEA	European Agency for the Evaluation of Medicinal Products
EPI	epidermal powder immunization
EPO	erythro poietin
eqIL-2	equine interleukin 2
ESOPE	European Standard Operating Procedures for Electrochemotherapy and Electrogenetherapy
FDA	Food and Drug Administration (USA)
GAM	gene activated matrix
GCV	ganciclovir
GeMCRIS	Genetic Modification Clinical Research Information System
GFP	green fluorescence protein
GM-CSF	Granulocyte-macrophage colony stimulating factor
GTAC	Gene Therapy Advisory Committee
GTEG (EMEA)	Gene Therapy Expert Group
GLP	good laboratory practice
GMO	genetically modified organism
GMP	good manufacturing practice
GTA	gene transfer agent
GT-MP	gene therapy medicinal product
HA-2	hemagglutinin subunit 2
HCG	Human Genetic Commission
hFIX	human factor IX
HGF	human growth factor hepatocyte growth factor
hnRNP	heterogeneous nuclear ribonucleoprotein

hSeAP	human secreted alkaline phosphatase
HSV-TK/HSVtk	herpes simplex thymidine kinase
HV	high voltage
ICH	International Conference on Harmonisation
i.d.	intradermal
IFN-γ	gamma interferone
IgG1	immunoglobuline G1
i.m.	intramuscular
IND	investigational new drug
IPC	in-process control
KSG	Kommission Somatische Gentherapie (Germany)
LAL	*Limulus amebocyte* lysate
LIF	laser-induced fluorescence
LPD	lipid/polycation/DNA
LPS	lipopolysaccharide
LV	low voltage
MAR	matrix attached region
MART-1	melanoma antigens recognized by T cells 1
MC	muscle cell
MCB	master cells bank
MDI	metered dose inhaler
mEpo	murine erythropoietin
MHC	major histocompatibility complex
MMP-3	matrix metalloproteinase-3 gene
MTC	magnetic targeted carrier
NFκB	anti-apoptosis mediator
NIH	(US) National Institute of Health
NLS	nuclear localization sequence
NLS (Both Ch 5)	nuclear localization signal
NOAEL	no-observed-adverse-effect level
NPC	nuclear pore complex
nt	nucleotide
NT	neutralization test
OBA	Office of Biotechnology Activities
oc	open circular
ORF2	open reading frame 2
ori	origin of replication

PCR	polymerase chain reaction
PDE	permitted daily exposure
pDNA	plasmid DNA
PEG_{5000}	polyethylene $glycol_{5000}$
PEI	polyethylenimine
PLGA	poly(lactide-co-glycolid)
PLK1	polo-like kinase 1
RAC	Recombinant DNA Advisory Committee
SALT	skin-associated lymphoid tissue
SCA1	spinocerebellar ataxia type 1
SCID (mice)	severe combined immune deficiency
shRNA	short hairpin RNA
siRNA	small interfering RNA
SOP	standard operating procedure
SV40	simian virus 40
TCR	T cell receptor
T_E cell	effector T cell
T_H cells	helper T cell
Th-1/2	T helper 1/2
TNF-α	tumor necrosis factor (α)
TLR	Toll-like receptor
TSE	transmisible spongiform encephalopathy
VEGF	vascular endothelial growth factor
WCB	working cells bank

1
DNA Vaccines – An Overview

Britta Wahren and Margaret Liu

1.1
Rationale for DNA Vaccines

Administration of genes via DNA or RNA may be considered the next-generation of scientific development following the use of recombinant proteins for prophylactic vaccines or for therapy. The use of DNA vaccines for the generation of immune responses arose from efforts to find immunogens that would be able to overcome some of the limitations of other modalities of vaccination. With the discovery of the potential widespread applications of DNA plasmids came appreciation of certain of the characteristics of DNA as a product: namely, its advantages, relative to other biologicals, for manufacturing (Chapter 3), product characterization, storage (Chapter 3), and delivery (Chapters 5–12).

From the standpoints both of therapeutics and of vaccines, the use of DNA arose from the desire to have a protein be produced *in situ*. For a variety of applications, ranging from cytokine administration to gene therapy for metabolic and inherited disorders, it was clear that administration of the gene rather than the protein could have multiple advantages: proteins synthesized *in situ* from DNA could potentially persist locally or systemically for longer periods of time without the toxicities associated with the high levels of intravenously administered proteins, certain proteins such as cytokines could be administered to the desired site (i.e., intratumorally) (Chapter 7) more readily when administered as genes, and a protein synthesized from the gene would have mammalian posttranslational modifications, thus avoiding one of the significant challenges that can arise when making recombinant proteins in nonmammalian hosts.

Although vaccines have been considered perhaps the greatest human health achievement, being successful even to the point of eliminating an entire wild-type disease from the planet (smallpox), certain diseases have remained unconquered by vaccination. Two key reasons for this are that the traditional approaches have either simply not worked, or have been considered potentially too risky for a disease such as HIV. As an example, although live attenuated virus vaccines have been extremely effective against a variety of diseases, they have at least the theoretical

risk of reversion to wild type, which in the case of HIV would render the vaccinee infected with a virus that causes what today is still a fatal infection.

As understanding of immune responses to disease increased, it became clear that the use of vaccines that induced primarily antibody responses might not be able successfully to target diseases that required a strong CD8+ T cell responses. Proteins that enter the cellular processing pathway resulting in the generation of CD8+ T cell responses generally have to be endogenously synthesized within a cell. Means to deliver the gene for an antigen, rather than the antigen itself, directly into cells were therefore sought, as the latter would generally result in the exogenous protein being taken into the endolysosomal processing pathway, with the resultant generation of MHC Class II-restricted CD4+ T cells rather than CD8+ T cells. The observation that plasmid DNA could directly transfect cells *in vivo* [1] came as a surprise given the complexity of viral structures that are designed for infecting cells. The process of DNA transfection is very inefficient and, moreover, the best transfected cell type is the muscle cell. Myocytes lack the immune accessory surface molecules needed to activate immune-responding cells appropriately, so it was a surprise to find that direct transfection of myocytes by immunization with unformulated plasmid DNA could indeed result in the generation of CD8+ T cells and protection against a lethal viral challenge [2].

DNA vaccines had further appeal as a product, in additional to their immunologic rationale. The manufacturing process promised to be fairly generic in comparison with those for other biologicals. Traditional live virus vaccines require years of challenging work to attenuate the pathogen properly and to design a cellular production system. Even recombinant proteins can be challenging, because of the need to find the correct producer cell able to make the antigen in the correct form (such as with the correct folding or posttranslational modifications). Because DNA vaccines are bacterial plasmids, the production is quite similar for different vaccines because they differ only in the gene sequence encoding the antigen. The majority of the plasmid, such as the backbone, can be identical or similar. Moreover, DNA vaccines at their simplest, being just plasmids, are potentially more stable (Chapter 3) than live viruses, an attribute that should facilitate their use in resource-poor settings.

1.2 Preclinical Proof of Concept

The initial demonstration that direct immunization with a simple plasmid of DNA encoding a protein from a pathogen could not only result in the generation of both arms of the immune response (cytotoxic T lymphocytes as well as antibodies), but could also protect from an otherwise lethal challenge [2] opened up the field of DNA vaccines. The ability to protect animals from a strain of virus different from the strain from which the gene was cloned generated considerable interest because it offered a potential means to make vaccines for diseases that have multiple strains, such as influenza or HIV. The influenza vaccine, for example, has to contain antigens

for three strains and needs to be reformulated each year as new strains arise. Not only is this a cumbersome process making the adequate yearly supply of vaccines problematic, but such a vaccine does not protect against the epidemic strains differing from the strain in the vaccine that occasionally arise mid-season. Of even more concern is the fact that such a vaccine will not protect against novel pandemic strains of influenza that periodically may arise, most notably in the 1919 Spanish influenza that killed millions of people worldwide. The demonstration that a DNA vaccine made from the genetic sequence of one strain was able to protect against challenge not just with a slightly different drifted strain, but against a different subtype, raised hopes for the ability of DNA vaccines to be effective against a variety of diseases.

From those initial studies, the scientific literature rapidly grew to thousands of publications demonstrating the ability of DNA vaccines to induce immune responses and protective and therapeutic benefits in a variety of preclinical disease models. These models not only included various infectious diseases, including those caused by viruses, bacteria, and parasites, but also encompassed other types of disease, such as cancer, allergy, and autoimmunity (reviewed in [3, 4]). Additional applications for autoimmune diseases and allergies are based upon the ability of the DNA to alter the type of generated T cell help specifically for the particular protein antigen. Autoimmune responses are thought to be due to the inappropriate overproduction of either T helper 1- or T helper 2-type responses. In animal models, DNA vaccines have been shown to be able to alter the form of T cell help, and DNA vaccines have thus been able to prevent or ameliorate the disease in preclinical models of asthma [5] and diabetes [6].

It soon became evident, however, that DNA vaccines, while robust in small animal models, were less immunogenic in nonhuman primates and humans (reviewed in [3, 4]). This has given rise to a variety of approaches for making DNA vaccines of increased potency, as is explored below.

1.3 Clinical Trials

Clinical trials have been performed for DNA vaccines encoding antigens from pathogens and tumors. In addition, however, trials have been performed with DNA encoding therapeutic proteins where not an immune response, but rather expression of the therapeutic protein, is desired. Such studies have included the therapeutic administration of a gene encoding a normal growth factor such as Fibroblastic Growth Factor, or other growth factors, the intent being not to replace a defective or missing protein, but rather to administer a supraphysiologic amount of the growth factor to a local site for a period of time more prolonged than would be achievable by administration of the recombinant protein [7, 8]. The factor then induces the growth of new blood vessels to ameliorate the ischemic condition of the limb or myocardium. DNA has also been used for what is more traditionally considered to be the purview of gene therapy: DNA encoding a form of the muscle