

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 Schlee: “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

Contents

Preface	V
List of Contributors	XV
Abbreviations	XIX
1 DNA Vaccines – An Overview	1
<i>Britta Wahren and Margaret Liu</i>	
1.1	Rationale for DNA Vaccines 1
1.2	Preclinical Proof of Concept 2
1.3	Clinical Trials 3
1.4	Second-Generation Vaccines 4
1.5	Conclusions 5
	<i>References 5</i>
2 DNA as a Pharmaceutical – Regulatory Aspects	7
<i>Carsten Kneuer</i>	
2.1	Introduction 7
2.2	Quality Requirements for DNA used as a Gene Therapy Product 9
2.2.1	Introduction 9
2.2.2	Production and Purification 9
2.2.2.1	Raw Materials 9
2.2.2.2	Antibiotics 10
2.2.2.3	Solvents 10
2.2.2.4	Fermentation 10
2.2.2.5	Purification 11
2.2.3	Cell Banking System Procedures 11
2.2.3.1	Generation and Characterization of Master and Working Cell Banks 11
2.2.4	Product Characterization and Quality Criteria 12
2.2.4.1	Identity 13
2.2.4.2	Purity 13

2.2.4.3	Adventitious Agents	13
2.2.4.4	Potency	13
2.3	Safety Studies for Clinical Trials	14
2.3.1	General Considerations	14
2.3.2	Conduct of Preclinical Safety Studies	15
2.3.2.1	Regulations	15
2.3.2.2	Design of an Appropriate Toxicology Program	16
2.3.2.3	Single- and Repeat-Dose Toxicity Studies	16
2.3.2.4	Safety of the Formulated Plasmid DNA	16
2.3.2.5	Specific Safety Considerations	17
2.3.2.6	Choice of Animal Model	17
2.4	Special Issues	18
2.4.1	Comparability of Plasmid Gene Therapy Products	18
2.4.2	Mixed Plasmid Preparations	18
2.4.3	Plasmid Molecular Structure	19
2.5	Biosafety Issues and Environmental Risk Assessment	19
	<i>References</i>	20
3	From Bulk to Delivery: Plasmid Manufacturing and Storage	23
	<i>Carsten Voß, Torsten Schmidt, and Martin Schleaf</i>	
3.1	Introduction	23
3.1.1	Gene Therapy	23
3.1.2	DNA Vaccination	24
3.2	Manufacturing of Plasmid DNA	24
3.2.1	Bacterial Cultivation	24
3.2.2	Plasmid DNA Purification	26
3.2.3	Innovative Aspects in Plasmid Manufacturing	28
3.3	Quality Control of Plasmid DNA Vectors	30
3.3.1	Proteins, Ribonucleic Acid, and Lipopolysaccharides	31
3.3.2	Chromosomal DNA	31
3.3.3	Plasmid Identity	32
3.3.4	Plasmid Topology (Structural Homogeneity)	32
3.4	Plasmid Stability during Storage and Application	33
3.4.1	Long-Term Stability of Plasmid DNA	33
3.4.2	Lyophilization for Long-Term Storage	36
3.4.3	Stability during Application	37
3.5	Future Developments	37
	<i>References</i>	38

4	Minimized, CpG-Depleted, and Methylated DNA Vectors: Towards Perfection in Nonviral Gene Therapy	43
	<i>Oleg Tolmachov, Richard Harbottle, Brian Bigger, and Charles Coutelle</i>	
4.1	Introduction	43
4.2	The Mammalian Immune System as a Barrier to Nonviral Gene Delivery	44
4.3	Strategies to Minimize DNA Vectors	45
4.3.1	Excision of a DNA Fragment Containing a Transgene Expression Cassette from Plasmid DNA	46
4.3.2	Intramolecular Site-Specific Recombination Within a Bacterial Plasmid	46
4.3.3	Synthesis of Minimized DNA Vectors by PCR	48
4.3.4	Improvement of Minimized DNA Vector Yield and Purity	49
4.4	Depletion of CpG Dinucleotides in the Bacterial Vector Backbone	50
4.5	Methylation of CpG Dinucleotides in Plasmid DNA	50
4.6	Towards an Ideal Nonviral Vector	51
4.7	Conclusion	52
	<i>References</i>	52
5	Localized Nucleic Acid Delivery: A Discussion of Selected Methods	55
	<i>Christian Plank, Franz Scherer, and Carsten Rudolph</i>	
5.1	Foreword	55
5.2	Nucleic Acid Delivery – What For?	55
5.3	Nucleic Acid Delivery – How?	57
5.3.1	Nucleic Acid Compaction	58
5.3.2	Receptor–Ligand Interactions	59
5.3.3	Endocytosis and Endosomal Escape	59
5.3.4	Nuclear Transport	61
5.3.5	Genome Organization	61
5.3.6	Biocompatibility	62
5.4	Why is Localization of Drug and Nucleic Acid Delivery Important?	63
5.5	Hierarchies of Localization (Targeting)	65
5.5.1	Methods of Localization and of Local Control	66
5.5.2	Nuclear Transport of Macromolecules in Living Cells	68
5.5.3	Nuclear Localization Signals and Gene Transfer	70
5.5.4	Localization Hierarchies I and II – Establishing Target Cell Contact	73
5.5.5	Vector Localization by Magnetic Force (Magnetofection)	75
5.5.6	Hydrodynamic Methods of Nucleic Acid Delivery	80
5.5.7	Local Vector Implantation. Carrier-Mediated Nucleic Acid Delivery	81
5.5.8	Injectable Implants for Localized Nucleic Acid Delivery	87
5.5.9	Aerosol Application of Nucleic Acids	88
5.5.10	Use of Ultrasound to Trigger Localized Delivery	90
5.6	Concluding Remarks	92
	<i>References</i>	93

6	DNA Needle Injection	117
	<i>Matthias Giese</i>	
6.1	From Mouse to Human	117
6.1.1	DNA Vaccines	117
6.1.2	Successful Strategy for Vaccination	119
6.2	Intramuscular Injection	120
6.2.1	Biology of Muscle Fibers	120
6.2.1.1	Resting Stem Cells	120
6.2.2	Uptake of Plasmid DNA	121
6.2.3	Activation of the Immune System	121
6.2.3.1	Receptors and other Signals	122
6.2.3.2	Antigen Presentation	122
6.2.4	Cross-Priming	123
6.2.5	Safety Aspects	124
6.2.5.1	Uptake of the DNA by Muscle Cells	124
6.2.5.2	Antigen Processing	124
6.2.5.3	Antigen Presentation	125
6.2.6	DNA Vaccination of Horses against Infection with Equine Arteritis Virus I	126
6.3	Intradermal Injection	128
6.3.1	Skin-Associated Lymphoid Tissue (SALT)	128
6.3.2	DNA Vaccination of Horses Against Infection with Equine Arteritis Virus II	129
6.4	Concluding Remarks	131
	<i>References</i>	131
7	Needleless Jet Injection of Naked DNA for Nonviral in vivo Gene Transfer	133
	<i>Wolfgang Walther and Ulrike Stein</i>	
7.1	Introduction	133
7.2	In vivo Application of Jet Injection	136
7.2.1	Intratumoral Jet Injection of Naked Plasmid DNA	136
7.2.2	Analysis of Reporter Gene Expression in Jet-Injected Tumors	137
7.2.3	Analysis of the Stability of Jet-Injected Naked DNA	138
7.3	Conclusions	139
	<i>References</i>	140
8	Plasmid Inhalation: Delivery to the Airways	145
	<i>Lee A. Davies, Stephen C. Hyde, and Deborah R. Gill</i>	
8.1	Introduction	145
8.2	Delivery Methods	146
8.2.1	Lung Delivery by Instillation	146
8.2.2	Delivery by Aerosol	147

8.2.3	Aerosol Deposition	148
8.2.4	Aerosolization Devices	148
8.2.4.1	Metered Dose Inhalers	149
8.2.4.2	Dry Powder Inhalers	150
8.2.4.3	Nebulizers	150
8.2.5	Aerosolization of Plasmid DNA	152
8.2.6	Plasmid DNA/Lipid Complexes	153
8.2.6.1	Optimization of Aerosol Formulation	153
8.2.6.2	Aerosol Delivery of Lipid/pDNA to Human Lung	154
8.2.7	Plasmid Delivery with Cationic Polymers	155
8.3	Future Directions	157
8.4	Conclusions	158
	<i>References</i>	159
9	Hydrodynamic Gene Delivery	165
	<i>John W. Fabre</i>	
9.1	Definition	165
9.2	Initial Discovery of the Technique	165
9.3	The Systemic Hydrodynamic Approach	166
9.4	The Regional Hydrodynamic Approach to the Liver	167
9.5	Gene Delivery to the Liver in Large Animals	167
9.6	Hydrodynamic Gene Delivery to Tissues other than Liver	168
9.6.1	Skeletal Muscle	168
9.6.2	Kidney	169
9.7	Mechanisms of Gene Delivery	170
9.8	Safety and Clinical Applicability	170
	<i>References</i>	171
10	DNA Pharmaceuticals for Skin Diseases	173
	<i>Vitali Alexeev and Jouni Uitto</i>	
10.1	Introduction	173
10.2	Recombinant DNA-Based Skin Gene Therapy	174
10.2.1	Correction of Genetic Disorders	174
10.2.2	“Suicide” Gene Therapy	176
10.2.3	Genetic Pharmacology	176
10.3	DNA Vaccines	176
10.3.1	DNA Vaccination Through Skin	178
10.3.2	DNA Vaccines Against Skin Cancers	179
10.4	Physical Methods of DNA Delivery	180
10.4.1	Delivery of DNA to the Skin by Particle Bombardment	181
10.4.2	Microparticles for DNA Delivery	182
10.4.3	Genetic Immunization by Jet Injection	182
10.4.4	Epidermal Powder Immunization	183
	<i>References</i>	184

11	Electrotransfection – An Overview	189
	<i>Capucine Trollet, Pascal Bigey, and Daniel Scherman</i>	
11.1	Theory and Mechanisms	190
11.1.1	History	190
11.1.2	Mechanism of in vitro Electrotransfection at the Scale of a Single Cell	190
11.1.2.1	Permeabilization	190
11.1.2.2	Uptake of DNA	192
11.1.3	Mechanism of in vivo DNA Electrotransfer	192
11.2	In vivo DNA Electrotransfer in Practice	194
11.2.1	Device and Electrical Parameters	195
11.2.2	DNA Electrotransfer and Toxicity	197
11.2.3	Plasmid Biodistribution	197
11.3	Targeted Tissues	199
11.3.1	Skeletal Muscle	199
11.3.2	Tumor Tissue	200
11.3.3	Skin	201
11.3.4	Liver	201
11.3.5	Lung	202
11.3.6	Vasculature	202
11.3.7	Eye	202
11.3.8	Embryos	203
11.3.9	Cartilage	203
11.3.10	Gonads	203
11.4	Therapeutic Applications	204
11.4.1	Intramuscular Electrotransfer	204
11.4.1.1	Ectopic Secretion of Proteins	204
11.4.1.2	Muscle Disease Therapy	205
11.4.2	Vaccination	205
11.4.3	Cancer Gene Therapy	206
11.4.3.1	Strengthening Antitumor Response	207
11.4.3.2	Suicide Genes	207
11.4.3.3	Apoptosis-Inducing Genes	207
11.4.3.4	Inhibition of Tumor Angiogenesis	208
11.4.3.5	Other Strategies	208
11.4.4	Electrotransfer as a Tool	208
11.5	Conclusion	209
	<i>References</i>	210

12	Electrogenetransfer in Clinical Applications	219
	<i>Lluís M. Mir</i>	
12.1	Summary of the Basis of Electrogenetherapy	219
12.1.1	Tissue Electropermeabilization	219
12.1.2	DNA Electrophoresis	220
12.1.3	The Interest of Electrogenetherapy	220
12.2	The Road to Clinical Electrogenetherapy	221
12.2.1	Basic Difficulties and Requirements	221
12.2.1.1	Electrogenetherapy is a Local Treatment	221
12.2.1.2	DNA Injection	222
12.2.1.3	Need for Appropriate Electrodes	222
12.2.1.4	Need for Appropriate Electrical Pulse Generators	222
12.2.1.5	Electrogenetherapy and Public and Professional Perceptions of the Biomedical Use of Electricity	222
12.2.2	The Cliniporator Project	223
12.2.3	The ESOPÉ Project	223
12.2.4	Future Perspectives	224
	<i>References</i>	225
13	Cancer Inhibition in Mice After Systemic Application of Plasmid-Driven Expression of Small Interfering RNAs	227
	<i>Birgit Spänkuch and Klaus Strebhardt</i>	
13.1	Introduction	227
13.2	Plasmid-Expressed siRNA	228
13.2.1	PLK1 shRNA-Mediated Inhibition of PLK1 Expression	228
13.2.2	Nuclease Inhibitor ATA and Stability of Plasmid DNA in Mammalian Blood	230
13.2.3	Antitumor Activity of PLK1 shRNA in vivo	232
13.2.4	Vector-Induced Decreased Expression of PLK1 and Antitumor Activity	234
13.3	Conclusion and Future Directions	237
	<i>References</i>	238
	Subject Index	241

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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 megalovirus
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.	intra-dermal
IFN- γ	gamma interferone
IgG1	immunoglobuline G1
i.m.	intra-muscular
IND	investigational new drug
IPC	in-process control
KSG	Kommission Somatische Genterapie (Germany)
LAL	<i>Limulus ameobocyte</i> 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
NF κ B	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	transmissible spongiform encephalopathy
VEGF	vascular endothelial growth factor
WCB	working cells bank

1

DNA Vaccines – An Overview

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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 addition 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