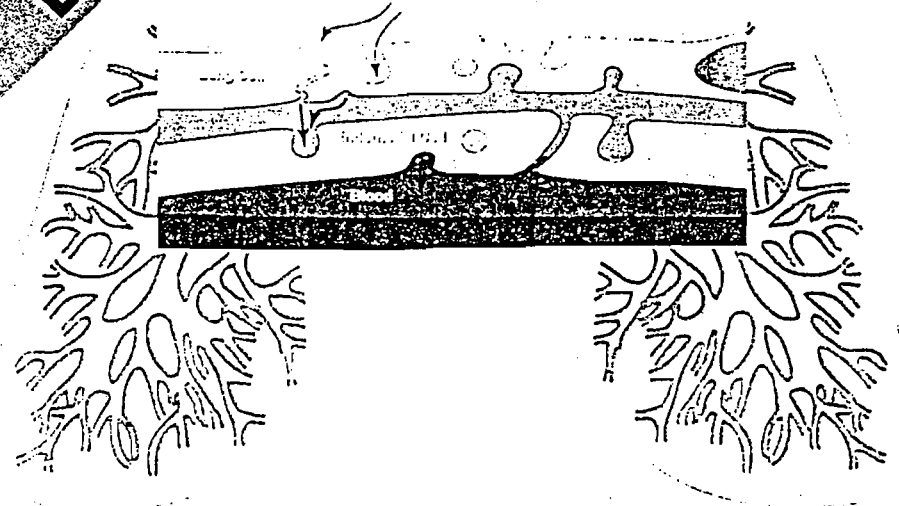
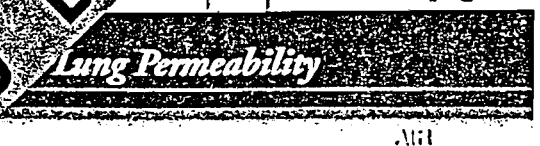
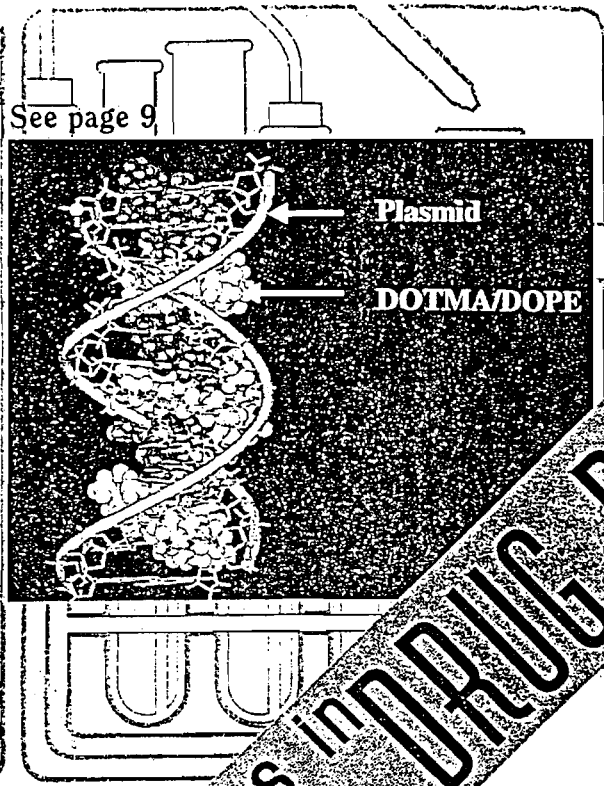
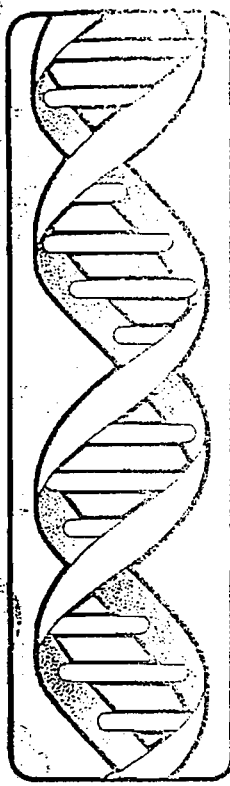


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Innovations in DRUG DELIVERY

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Nonviral Gene Therapy

From Bench to the Clinic

R. I. Mahato, M. P. Fons, A. Rolland

Why Nonviral Gene Therapy?

The body contains a plethora of proteins whose absence or overproduction can lead to a variety of clinical manifestations depending on the structural

or functional role that the proteins normally play in the body [1]. Gene therapy is a method for the prevention, correction or modulation of genetic and acquired diseases that uses genes to provide the patient's somatic cells with the genetic information necessary to produce specific therapeutic proteins. The use of plasmid-based gene medicines is intended to overcome the limitations associated with the clinical use of protein drugs, including their low bioavailability, poor pharmacokinetics, chemical instability and relative high cost. In addition, gene therapy has the unique ability to affect the intracellular distribution of the expressed protein in defined compartments (e.g., mitochondria or cell membranes) and to direct antigens to a specific pathway (MHC class I or class II) in order to modulate a preferred immune response. Compared to viral vectors, gene medicines present several potential advantages, including: low cost, non-infectivity, absence of immunogenicity, good

compliance, and possibility of repeated clinical administration [2].

Gene Expression Systems

A gene medicine contains three components: a therapeutic gene that encodes a specific therapeutic protein; a plasmid-based gene expression system that controls the functioning of a gene within a target cell, and a gene delivery system that controls the delivery of the plasmid expression system to specific locations within the body. A gene expression system contains a plasmid backbone, gene(s) and genetic elements [3]. The gene expression system can be engineered to control whether the resulting

protein will remain within the cell for an intracellular effect or will be secreted out of the cell for either a local or systemic action. The gene expression system can also be adjusted to control the level of protein production, as well as the fidelity and duration of gene expression (Figure 1) [4].

Gene Delivery Systems

One of the major challenges in effective gene delivery resides in the ability to circumvent numerous barriers that a plasmid will encounter from its administration site to the nucleus of the target cell. These include: (i) rapid DNA degradation in blood or tissues by nuclease; (ii) limited dispersion of DNA in interstitial or intracellular spaces; (iii) inability of DNA to cross intact basement membranes of the endothelium or epithelium; (iv) inefficient uptake by target cells; and (v) poor translocation of DNA across periplasmic or nuclear membrane [5]. Each biological target will require a unique combination of delivery elements to overcome these key-limiting steps in the overall gene transfer *in vivo*.

Gene delivery systems are designed to control the location of a gene within the body by affecting the distribution and access of a gene expression system to the target cell and/or recognition by a cell-specific receptor followed by intracellular trafficking and nuclear translocation. To date a number of gene delivery systems have been developed, including lipids, polymers, peptides, and lipopeptides [6-16]. Some special devices, such as microinjection, electroporation and particle bombardment, have also been developed for introducing genes to specific targets [17, 18]. Among them, cationic lipid-based systems have reached the clinic and are the most widely investigated for gene delivery to the endothelium, pulmonary epithelium and tumor cells. Gene delivery and expression are affected by the route of administration, the biological target, the lipid/plasmid charge ratio, cationic lipid/co-lipid molar ratio, liposome size, methods of formulation and the amount of DNA [6-8]. For site-specific gene delivery, polymers and peptides, such as poly(L-lysine), histone, protamine, polyethyleneimine (PEI), dendrimers and lipopeptides, are linked to a cell-specific ligand, then bound to plasmid via electrostatic interactions. The resulting complexes retain their ability to interact specifically with target cell

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receptors [13, 15, 19]. However, no clinical study using cationic polymers or peptides has been reported to date.

In order to search for optimal delivery systems and methods, research will continue to address several rate-limiting steps, such as cellular uptake, release from endosomes, dissociation of the DNA from the delivery carrier in the cytoplasm or nucleus (dependent upon delivery system) and nuclear uptake.

Clinical Trials

Gene therapy clinical trials aim at answering the crucial questions related to the safety and efficacy of a gene therapy product. To date approximately 12 clinical trials have reported data on human subjects treated with nonviral gene therapy. Most of these studies use cationic lipid/plasmid complexes as a delivery system, the remainder use unformulated plasmid in saline (so-called 'naked DNA').

Direct intratumoral injection of lipid/plasmid complexes has been reported in a number of studies. Nabel et al. [20, 21] reported expression of the gene encoding a foreign major histocompatibility complex protein, HLA-B7, in melanoma patients receiving cationic lipid (DMRIE:DOPE)/plasmid complexes. Positive biological and therapeutic responses were observed as indicated by an increase in HLA-B7 specific CTL activity and regression of melanoma nodules. Rubin et al. [22] employed the same cationic lipid-based system (DMRIE:DOPE) containing the combination of the HLA-B7 gene and β -microglobulin (Allovectin-7, Vical Inc., San Diego) for the immunotherapy of hepatic metastases of colorectal carcinoma. The HLA-B7 protein was detected in 5 out of 8 patients by immunohistochemistry and 7 out of 14 patients by fluorescence-activated cell sorting analysis. In a phase II clinical

trial, treatment of melanoma patients with lipid/HLA-B7 expression plasmid complexes resulted in disease stabilization in 6 out of 38 patients and partial tumor regression in 2 patients. In addition, intratumoral administration of cationic lipid/IL-2 expression plasmid complexes resulted in clinical benefit in 4 out of 14 renal cell carcinoma patients [23]. In a different clinical trial, positive biological response was also reported in 3 out of 3 cancer patients receiving intratumoral injection of cationic lipid (DC-Chol) formulated with E1A gene expression construct (Targeted Genetics Corporation, Seattle). In a follow-up study, 11 out of 16 patients had either stable disease or tumor regression after intratumoral injection of the cationic lipid/E1A gene complex [24].

Cationic lipid/plasmid complexes were used to deliver the cystic fibrosis transmembrane conductance (CFTR) gene to the nasal epithelium of cystic fibrosis patients [25]. Partial restoration of chloride conductance was observed in 2 out of 9 patients, suggesting a positive pharmacological response. Brigham et al. [26] demonstrated expression of human α -antitrypsin (AAT) and significant decrease in interleukin-8 (IL-8) levels in the treated nostril of all five patients after nasal administration of cationic lipid system (DOTMA:DOPE)/AAT plasmid complexes. This anti-inflammatory effect was not observed after systemic administration of the purified hAAT protein (Prolastin™), even though physiologic concentrations and nasal lavage (BAL) concentrations of AAT were achieved. This points out the potential advantage of gene therapy approach over the use of protein drugs, and the possibility of generating a new quality of pharmacological response by expression of a protein at the desired location for a sustained period of time.

In a recent Phase I vaccination trial [27], intramuscular injection of 'naked DNA' produced a good cellular immune response to malaria antigen in 20 volunteers. Intramuscular injection of 'naked DNA' encoding vascular endothelial growth factor (VEGF) resulted in gene expression and therapeutic benefit in 7 out of 9 patients with peripheral limb ischemia, as measured by increased peripheral circulation [28].

Commercial Challenges

Fundamental commercial challenges facing gene medicines as they proceed to the market will be to provide therapeutic benefit within the confines of an acceptable safety profile. Major advances in genetics and the ability to

Figure 1—Spatial and temporal modulation of gene expression

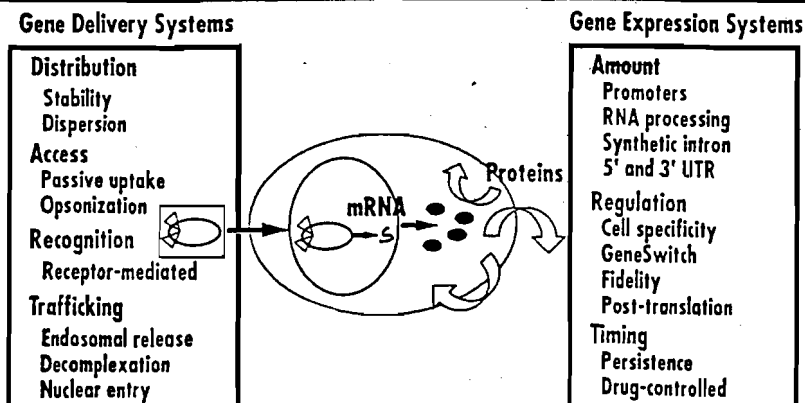
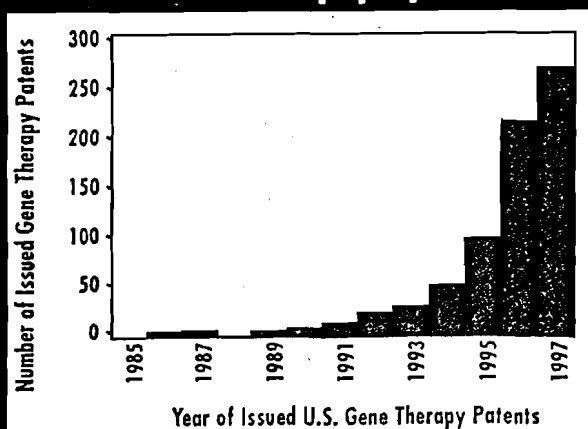


Figure 2—Rapid growth of gene therapy intellectual property



control gene delivery and expression will bring revolutionary therapeutic methods in the upcoming millennium. Rapid technological development may produce potential products or technologies, which could become obsolete before a given company recovers its

research, development and capital expenditures. Furthermore, numerous patents are being issued that cover the broad concepts of technology (Figure 2), which can inhibit the development of new technologies that are directly applicable to a product [29].

Gene medicines are still in an early phase of clinical development and there are currently no marketed gene therapy products. Each therapeutic product containing a particular gene will likely be regulated as a separate biological product, depending on its intended use and the Food and Drug Administration (FDA) policies in effect at such time. Regulatory requirements for gene therapy are constantly evolving and being updated [30].



Perspectives

Although expression of various therapeutic genes have been demonstrated in animal models, human clinical trials have not met all the expectations to date, with still few individual cases of success. With

the enthusiasm to proceed to clinical trials, basic studies have not always been given adequate attention. Major difficulties include shortcomings in most current gene delivery systems, inadequate understanding of the biological interaction with the host and poor intracellular trafficking of DNA plasmid [31]. Improvements in gene expression systems are also needed to increase the promoter activity and mRNA stability, while maintaining a safety profile, which avoids integration or homologous recombination.

Despite early setbacks, there is no doubt that gene medicines, which present the potential to be the most potent therapeutic molecules (Figure 3), will eventually be used as pharmaceuticals. At this crucial juncture, we must manage risk through teamwork and cross-disciplinary efforts at innovation [32]. The innovation comes in an environment where scientific freedom is held sacred and thus scientists should be given the full intellectual freedom they could enjoy in a corporate setting [33]. Because of scarce resources and tough competition, gene therapy companies must focus their efforts on projects with the greatest chance of scientific, medical and commercial success.

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Figure 3—Size and potency of pharmaceutical products

