
Preface

In the early 1990s, almost 200 yr after Edward Jenner demonstrated the effectiveness of the smallpox vaccine, a new paradigm for vaccination emerged. The conventional method of vaccination required delivery of whole pathogens or structural subunits, but in this new approach, DNA or genetic information was administered to elicit an immunological response. Once it was observed that plasmid DNA delivered *in vivo* led to production of an encoded transgene (1), two ground-breaking studies demonstrated that immunological responses could be generated against antigenic transgenes via plasmid DNA delivered by DNA vaccination (as this approach is called) (2,3). The appearance of this new vaccination strategy coincided with advances in molecular biology, which provided new tools to study and manipulate the basic elements of an organism's genome and also could be applied to the design and production of DNA vaccines.

DNA Vaccines is a major updated and enhancement of the first edition. It reviews state-of-the-art methods in DNA vaccine technology, with chapters describing DNA vaccine design, delivery systems, adjuvants, current applications, methods of production, and quality control. Consistent with the approach of the *Methods in Molecular Medicine* series, these chapters contain detailed practical procedures on the latest DNA vaccine technology.

The enthusiasm for DNA vaccine technology is made clear by the number of research studies published on this topic since the mid-1990s. Why the rapid growth of interest in DNA vaccines? First, DNA vaccines represent a simple and powerful concept: the coding sequence of an antigenic pathogen gene is incorporated into plasmid DNA, which will allow its expression in host cells. Thus, DNA vaccines circumvent the need for preparation, purification, and delivery of a pathogen or antigenic protein. Instead, they utilize the intrinsic machinery of host cells. Second, conventional vaccination approaches have failed to yield useful vaccines for a large number of infectious diseases and have made only modest progress in treating cancer. DNA vaccines may be able to engage immunological mechanisms that are not easily attainable with other approaches. Third, methods to produce, manipulate, and purify DNA are now standard in most biology and bioengineering laboratories, making the tools of DNA vaccine production widely accessible.

Investigators rely on recombinant DNA principles to generate plasmids with the promoter, antigenic coding regions, and other sequences necessary to achieve optimal expression in host cells. Many of these methods are described

in Part I. The resulting constructs must be administered to animals or humans to produce an immune response. Part II describes methods for DNA delivery, covering both the wide range of routes for DNA vaccine administration (including oral, topical, intradermal, intranasal, intramuscular, intratumoral, and intravenous), as well as many approaches for enhancing the efficiency of DNA delivery into cells (including microinjection, biolistic particle bombardment, electroporation, complexation with cationic lipid or polymer preparations, and incorporation in nano- or microparticles). Although our understanding of mechanisms underlying the initiation of immunological responses after DNA vaccine delivery is imperfect, numerous methods for enhancing vaccine activity have already been developed. Some of these approaches, including the use of adjuvants, are described in Part III. While there are already too many applications for DNA vaccine technology to be reviewed completely, Part IV contains illustrations of some key concepts including applications to allergy, avoidance of autoimmunity, and DNA vaccine responses in neonates and infants. Clinical applications of DNA vaccines are well underway; Part V reviews methods for DNA production and assurance of quality.

Over the past decade, promising DNA vaccination results in animal models have been translated into the clinic quickly, making DNA vaccination the most important early application of nonviral gene therapy. By 2004, 24% of gene therapy clinical trials involved nonviral vectors (4). To date, clinical trials have investigated the utility of plasmid DNA for vaccination against influenza, malaria, hepatitis B, human papillomavirus (HPV), and infectious diseases caused by HIV, as well as neoplastic diseases such as melanoma (5–13). The primary objective in Phase I clinical trials for DNA vaccines is to establish the safety and tolerance of the therapy. Clinical trials for DNA vaccines to date have employed a wide variety of doses (ranging from 0.25 to 2500 mg), delivery strategies (needle or needleless injection), administration sites (intramuscular or epidermal), carriers (naked, polymeric microparticles, or cationic liposomes), and dosing schedules. So far, DNA vaccines appear to be safe and well tolerated by patients, with mild side effects at the site of administration that are comparable to existing vaccination strategies.

DNA vaccines also appear to be capable of eliciting both humoral and cellular immune responses, leading to clinical effectiveness. For example, a Phase II clinical trial investigating precancerous cervical lesions from HPV retrospectively reported a statistically significant resolution of lesions in women younger than 25 yr (7). Unfortunately, in most studies the immune response to DNA vaccines has varied substantially between subjects. In the HPV trial, for instance, lesion resolution was not observed in the entire study population (7). Intramuscular administration of naked DNA, which has been successful in generating both humoral and cellular responses in animal models, has not been as

successful in humans. In one study, intramuscular injection of DNA stimulated strong cytotoxic T lymphocyte responses, but failed to induce detectable antigen-specific antibodies (8). In contrast, intramuscular injections of DNA encapsulated in microparticles or intraepidermal delivery of DNA were able to elicit both humoral and cellular responses (11,12). More work is needed to reconcile results from these disparate studies, but it seems clear that the route of DNA vaccine and the methods of vaccine preparation have strong effects on the immune response and the effectiveness of that response in preventing or treating disease.

One important clinical application of DNA vaccines may be to complement or augment traditional vaccines. For example, DNA vaccines have been tested in patients who failed to respond to conventional vaccinations for hepatitis B (10). The majority of subjects in one study developed a protective antibody response after DNA vaccination. It may also be advantageous in some settings to stimulate immunity with multiple vaccine preparations. To induce malaria immunity, for instance, subjects were primed with a DNA vaccine and then boosted with the recombinant antigen (13). This prime-boost approach was able to elicit both humoral and cellular immunity in subjects.

It is an exciting time for DNA-based vaccine technology, which has moved from pioneering animal studies to clinical testing quite rapidly. As the technology moves from the benchtop to the patient, industrial scientists and engineers are becoming even more important contributors. Though significant problems remain to be solved, we have made tremendous progress, as illustrated by the chapters in this volume. DNA vaccination offers a new platform technology for treatment and prevention of human disease with attributes that make it suitable for both developed and developing nations.

W. Mark Saltzman
Jeremy S. Blum

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