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REVIEW

# DNA vaccines: a review

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**Abstract.** Liu MA (Strasbourg, France and Karolinska Institute, Stockholm, Sweden). DNA vaccines: a review (Review). *J Intern Med* 2003; **253:** 402–410.

The DNA vaccines are simple rings of DNA containing a gene encoding an antigen, and a promoter/terminator to make the gene express in mammalian cells. They are a promising new approach for generating all types of desired immunity: cytolytic T lymphocytes (CTL), T helper cells and antibodies, whilst being a technology that has the potential for global usage in terms of manufacturing ease, broad population administration and safety. This review gives an overview of the mechanisms, preclinical and clinical efficacy of DNA vaccines, and point out the limitations of the first generation of such vaccines, and some of the promising second-generation developments. This technology is also being utilized in the field of proteomics as a tool to elucidate the function of genes. The breadth of applications for DNA vaccines thus ranges from prophylactic vaccines to immunotherapy for infectious diseases, cancer, and autoimmune and allergic diseases.

**Keywords:** DNA vaccines, infectious diseases, cancer, CTL (cytotoxic T lymphocyte), genes, clinical efficacy.

**List of Abbreviations**: CTL, cytolytic T lymphocyte; Th, T helper cell; MHC, major histocompatibility complex; Ab, antibody.

## Introduction

A well-known Chinese adage states, 'Give a man a fish and you feed him for a day. Teach a man to fish, and you feed him for a lifetime'. Whilst this has often been utilized in designing social assistance programmes, it is also the secret behind the incredible success of vaccines as a medical invention. Indeed, vaccines are considered amongst the most, if not *the* most, effective medical development because they have successfully eliminated an entire wild-type disease from the planet (smallpox) with a second disease about to be eradicated (polio). The secret behind this success lies to a large extent in the ability of vaccines to teach the body to respond to the wild-type pathogen, rather than directly treating the disease, as therapeutics such as antibiotics do.

## Need for new vaccines

A number of diseases have not yet been conquered by vaccines. Millions of people, including millions of children die each year from infectious diseases for which there is no effective vaccine. They include newly emergent diseases such as HIV/AIDS and ancient scourges such as malaria. Additionally, immunotherapeutic vaccines for certain diseases such as cancer are critically needed as therapies. It has been felt that the inability of previously existing technologies to develop the required vaccines is because of the different types of immune responses that has to be generated for certain diseases including the unique pathophysiological characteristics of those diseases. In addition, issues such as the manufacturing requirements for certain current

vaccines make the older vaccines less attractive technologies for a global scale. More recently, a new impetus has been added to the generation of new vaccines: bioterrorism. The threat of the misuse of infectious agents has created an urgency to develop new vaccines that have an increased safety profile and which can be easily administered to large populations.

## Immunological issues for vaccines

New efforts to develop vaccines emphasise inducing CD8+ cytolytic T lymphocytes (CTL) responses and antibodies because of the increasing recognition of the role and need for CTL in such vaccines. Likewise, efforts are being taken to develop vaccines that can induce specific types of T helper responses, Th1 or Th2. The traditional methods for developing vaccines are given in Table 1 which compares their characteristics with DNA vaccines. Examples of a live attenuated viral vaccine include vaccines for measles, mumps and rubella which are given as a combined vaccine. This vaccine is extremely effective, preventing at least 95% of children from all the three diseases. The efficacy of the vaccine in the US is shown by the decrease from 500 000 reported cases of measles per year before the licensure of the measles vaccine in 1963 [1] to only 86 cases reported in 1999 (including children who had not been immunized). Recombinant protein vaccines are also quite efficacious with an example being the licensed recombinant hepatitis B vaccines that have been shown to protect at least 95% of recipients. Although viral vectors and DNA vaccines have comparative attributes as given in Table 1, they are only in early stages of clinical development. Thus there are no examples of these types of vaccines as licensed products.

**Table 1** Comparison of vaccine technologies

| |
|---|
| Live attenuate viruses |
| Highly effective |
| Potential risk for certain ones |
| Manufacturing challenge |
| Recombinant proteins |
| Potent antibody response |
| Effective |
| Non-native forms at times |
| Not induce cytolytic T lymphocytes (CTL) |
| Viral vectors |
| Potential risk |
| Resistance/pre-existing antibody |
| Inflammation |
| DNA vaccines |
| Need for increased potency |
| Designer immune responses (e.g. type of helper T cell). |
| Specificity: avoid deleterious or diversional antigens |
| Relative stability |
| Safety |
| Generic manufacturing |
| Cost advantage |

Figure 1 illustrates, in a simplified form, the intracellular and intercellular interactions required for an antigen to result in the generation of both cytotoxic and helper T-cell responses, and antibody generation. The reason that recombinant protein or inactivated virus vaccines cannot generate the desired CTL response is that generally such a vaccine is taken up by an antigen-processing cell into the endolysosomal system, degrades into peptides and then associates with major histocompatibility complex (MHC) Class II molecules. These peptide/MHC complexes stimulate Th cells rather than the cytolytic T cells. In order to generate the CTLs, protein synthesized within a virally-infected cell enters a cellular processing pathway from the cytoplasm that results in peptides associating with MHC Class I molecules. These in turn are recognized by the appropriate cytolytic T cells that then can be activated to kill the infected cell [2]. Thus, if one could deliver a gene encoding an antigen into a cell (as a virus does during infection), the protein (in this case an antigen) following synthesis would be in the cytoplasm where some of it would enter the intracellular processing pathway resulting in the presentation of its relevant peptides on MHC Class I molecules for the stimulation of CTL.

The use of a live virus can be an effective means to accomplish this gene delivery with resultant CTL response. However, for certain viruses such as HIV, the use of a live virus, even attenuated, is considered too risky [3]. As AIDS is currently a fatal disease, there is a possibility that the attenuated virus vaccine strain could revert to the wild type or virulent strain as can occur for the oral polio virus vaccine. In addition, certain live viruses have developed specific mechanisms to elude or down-regulate the ensuing immune response. Many new technologies have been explored to specifically stimulate this MHC Class I-restricted CTL response

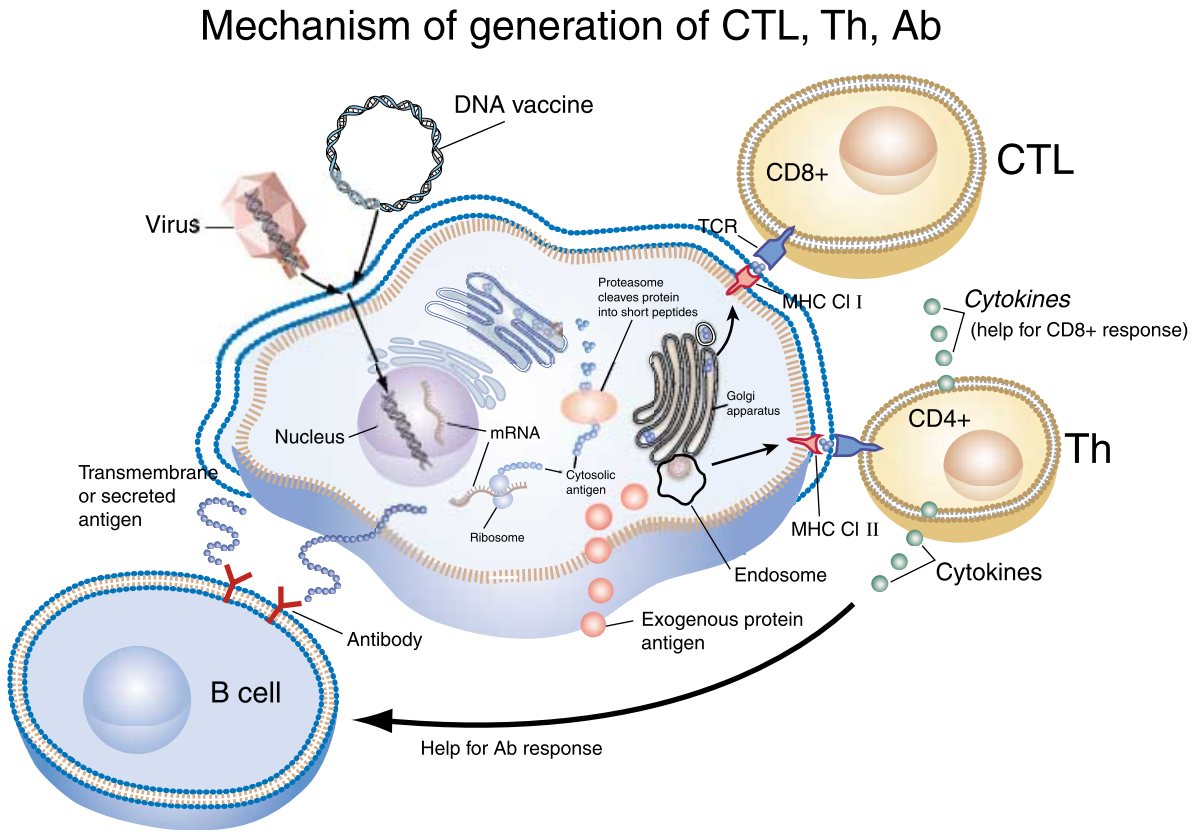


**Fig. 1** Depiction of the mechanisms of generation of antigen-specific humoural and cellular responses. Professional antigen presenting cells take up an exogenous antigen (e.g. a protein outside of the cell) into its endolysosomal degradation pathway. The protein is degraded to peptides that associate with major histocompatibility complex (MHC) Class II molecules that then are exhibited on the surface of the cell. Specific helper T cells (CD4+ T cells) recognize this antigen peptide/MHC Class II molecule complex and are activated to produce 'help' in the form of cytokines. These cytokines have myriad activities including, depending upon the cytokine, helping B cells activate into antibody-producing cells, and helping cytolytic T lymphocyte responses. Activation of cytolytic T lymphocytes (CD8+ T cells) generally is dependent upon an antigen-processing pathway reserved for intracytoplasmic proteins that are degraded into peptides that associate with newly synthesized MHC Class I molecules. These complexes, when presented on the surface of antigen presenting cells in conjunction with co-stimulatory molecules, result in the activation of the proper CD8+ T cells. For antibody responses, B cells recognize and respond to antigens that are either present extracellularly, or exposed extracellularly by being transmembrane proteins.

without the concerns and limitations inherent in a live attenuated virus vaccine.

## Characteristics of DNA vaccines

Viruses have highly evolved structures and mechanisms that enable them to introduce their genetic material into infected cells. Therefore, despite emerging evidence in the 1980s, it was not until a 1990 publication by Felgner and colleagues that the ability of simple plasmids of DNA (circular rings of DNA that exist extrachromosomally in bacteria) to directly enter mammalian cells when injected *in vivo* with ensuing synthesis of the protein they encoded [4] was accepted. The plasmid required no formulation or alteration other than a promoter active in mammalian rather than bacterial cells (see Fig. 2). DNA plasmids as gene delivery vehicles have a number of advantages over other systems (Fig. 3) which involve either removal of cells from an individual in order to transfect them *in vitro* prior to re-implantation of the transfected cells, or which require the manipulation of viruses and bacteria (which are themselves pathogenic, immunogenic or both) – a process significantly more complicated than manipulating and producing plasmids. But there were some concerns regarding their suitability and capability as vaccines. One of

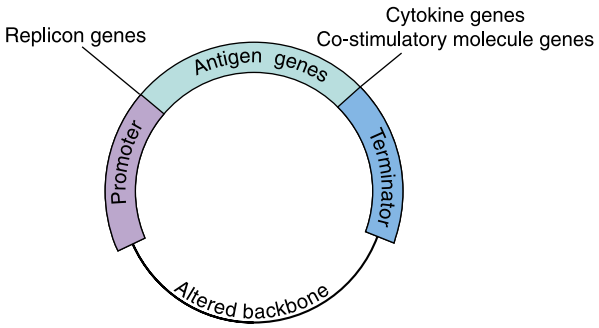


**Fig. 2** A schematic representation of a DNA vaccine. DNA vaccines are bacterially derived plasmids containing a gene encoding the desired antigen. Expression is driven by a promoter active in mammalian cells (generally a strong viral promoter), a transcription terminator, and often an antibiotic resistance gene that facilitates the selection of the plasmid during production in bacteria. Sites for increasing the potency of DNA vaccines are shown. For example, additional genes encoding cytokines or co-stimulatory molecules can be added to the gene for the antigen. Genes encoding a viral replicase has been shown to increase the potency of DNA vaccines. Alterations to the plasmid can also result in increased protein production, leading to increased immune responses.

them arose from the original observation by Felgner and colleagues that the *in vivo* transfection of cells was still an inefficient process [4]. Moreover, the cell type that took up the DNA and produced the encoded protein most efficiently were muscle cells, a cell type that under normal conditions is not involved in the generation of immune responses.

## Antigen presentation following DNA vaccination

As shown in Fig. 4(b), in order for a cell – which has synthesized an antigen – to successfully present antigen to a naïve T cell resulting in the activation of the cell, interaction between other molecules on the surfaces of the T cell and the antigen-presenting cell (known collectively as co-stimulatory molecules) must occur in addition to the recognition of the antigen/MHC Class I complex by the T-cell receptor. Muscle cells are not professional antigen-presenting cells, and thus lack the co-stimulatory molecules (Fig. 4a). Generally if a naïve T cell encounters a cell bearing the correct antigen–MHC Class I complex in the absence of the co-stimulatory molecules, then the T cell becomes unresponsive to the antigen in the future, rather than activated. Thus, despite the ability of muscle cells to take up plasmid DNA and synthesise the encoded antigen, it was not known whether the use of plasmid DNA would be effective for generating the desired CTL responses.

## Initial demonstration of capability of DNA vaccines

The initial publication by my colleagues and me [5] regarding the ability of plasmid DNA to result in the generation of CD8+ MHC Class I-restricted CTL following *in vivo* immunization with plasmid encoding an influenza protein, and of the ability of this CTL response to protect mice subsequently given an otherwise lethal challenge with influenza was thus considered to be a surprising demonstration of the capabilities of this approach. Subsequent work demonstrated that whilst the myocytes were transfected and produced antigen, the actual activation of T cells occurred because of cross-priming of professional antigen presenting cells [6–9] (Fig. 4c) and potentially the direct transfection of antigen-presenting cells (Fig. 4b).

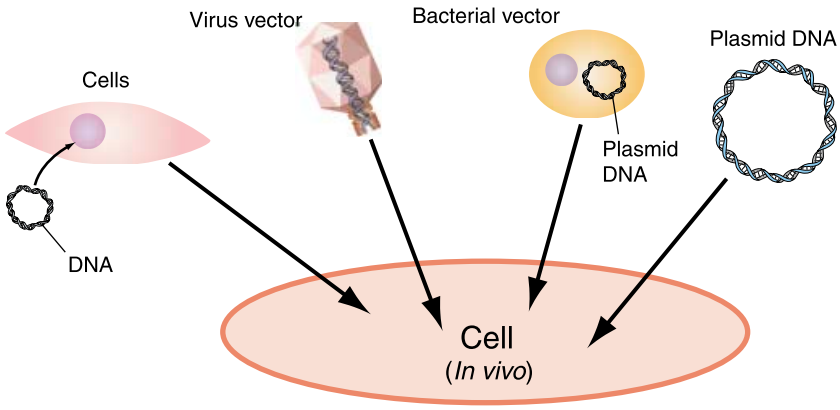


**Fig. 3** Various methods of gene delivery. Cells may be removed from the host, transfected *in vitro*, then re-implanted. Alternatively, a virus or bacteria can be modified such that it is no longer virulent, may be unable to replicate, and contains a gene encoding the desired antigen (and sometimes other viral/bacterial vector proteins). DNA vaccines are the simplest approach consisting of a plasmid encoding only the antigen.

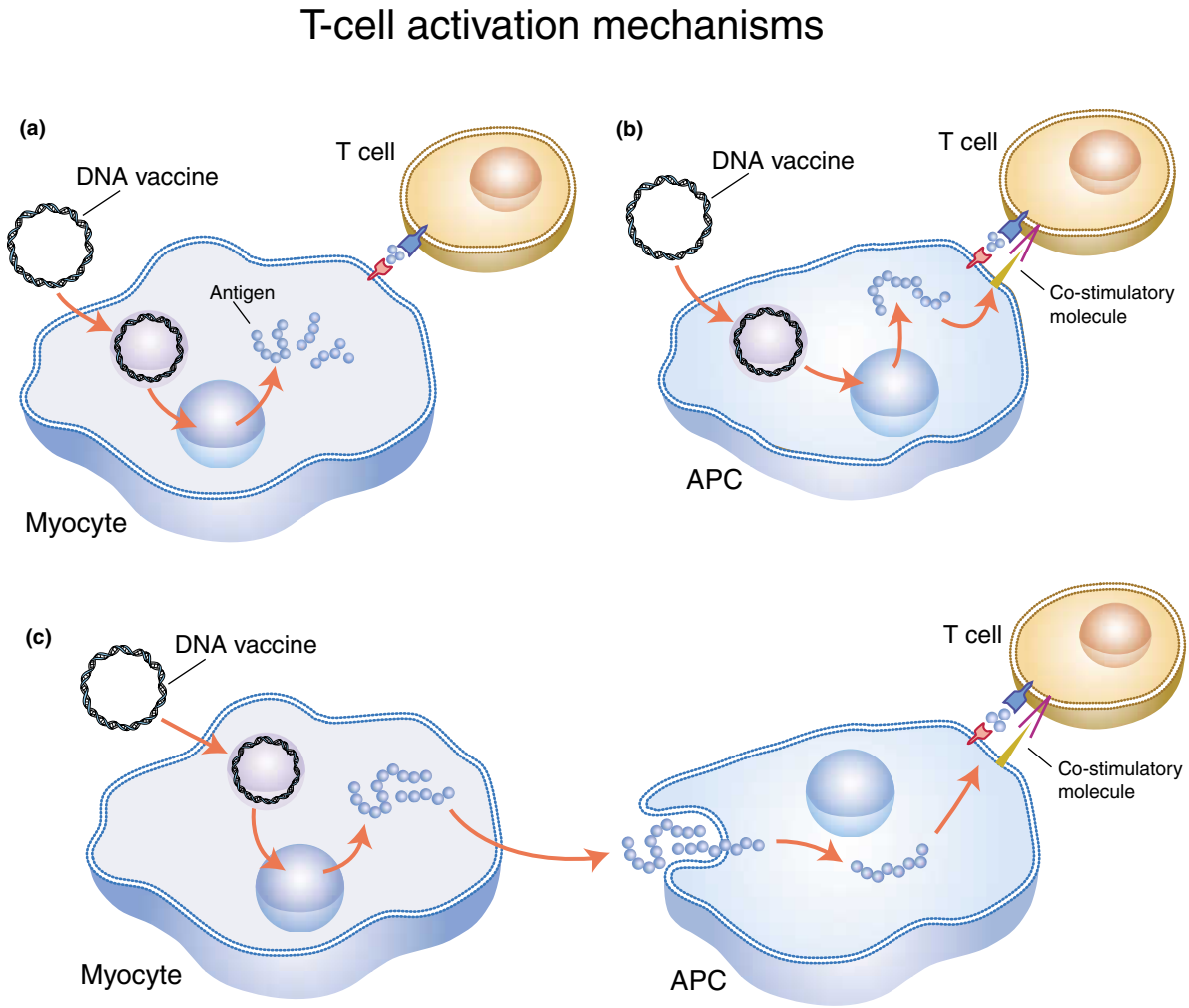


**Fig. 4** Potential cellular interactions whereby DNA vaccines result in the stimulation of CD8+ cytolytic T lymphocytes (CTL). Although muscle cells take up DNA and produce protein more than other cell types when DNA is injected *in vivo*, muscle cells usually lack the co-stimulatory molecules needed as part of the CTL activation process (a). The mechanism for activation of CTL following DNA immunization may involve direct transduction of professional antigen presenting cells (b). Another mechanism that has been demonstrated to occur is cross-priming wherein the muscle cell is transfected, produces the protein antigen, but then the antigen in some form is transferred to a professional antigen presenting cell which then is directly responsible for activating the CTL (c).

The protection observed in our initial work, was cross-strain, that is, the mice were protected from challenge with a strain of influenza that was of a different subtype from the strain from which the gene for the viral protein had been cloned. Influenza like HIV mutates easily and hence can easily escape the antibody-based immune responses induced by the existing influenza vaccines. Antibodies are generally most effective when directed against surface or envelope structures and some of these can easily mutate without adversely affecting the robustness of the virus. CTL responses can be directed at epitopes from any protein of the virus regardless of its location in the virus. As some of the internal or functional proteins would thus provide epitopes for CTL, a major strategy of vaccine development has been to develop CTL responses against conserved viral proteins in order to develop vaccines that would be effective against a broader range of strains of a virus. Hence the demonstration that a DNA vaccine could induce protection that was effective against a very different strain of virus (a different subtype of influenza, and one that arose 34 years later) than the strain from which the gene

was cloned opened the door for widespread development of DNA vaccines.

## Preclinical efficacy of DNA vaccines

A large number of scientists and clinicians worldwide have now demonstrated the preclinical immunogenicity and/or efficacy of DNA vaccines in disease models of infectious diseases, cancer, allergy and autoimmune diseases (Table 2) [10–12]. In the category of infectious diseases, the models have included viral, bacterial and parasitic diseases. The protection has been mediated by differing immune responses depending upon both the disease and the antigen. That is, CTL, antibodies and different types of Th responses have been generated. The role of the type of T cell that help in modulating immune responses is felt to be particularly important for the autoimmunity and allergic disease models.

## Clinical trials of DNA vaccines

The compelling preclinical results propelled DNA vaccines into clinical trials for a number of diseases: HIV (both as a prophylactic and an immunotherapeutic vaccine), malaria, influenza, hepatitis B and cancer. Whilst safety was demonstrated [13–15], and immune responses (both humoural [13, 14, 16–18] and cellular [16–20]) were generated, overall, the potency has been disappointing. Whilst most of the trials have utilized DNA vaccines injected intramuscularly, the hepatitis B DNA vaccine has been clinically tested by coating the DNA onto gold beads which are then propelled into the epidermis with a 'gene gun'. The so-called 'gene gun' was actually the first means whereby, in an animal model DNA was shown to be capable of *in vivo* delivery of a gene resulting in the generation of an antibody response [21]. This device propels gold beads coated with DNA directly into the epidermal cells and immune responses have been demonstrated in a variety of systems [22, 23]. In clinical trials with a gene gun, all vaccinees immunized with DNA encoding a hepatitis B antigen seroconverted, even those who had not responded to the licensed recombinant protein vaccine [17, 24].

Interestingly, certain HIV-infected patients responded to HIV DNA vaccines with antibody [16] or CTL [25] responses against antigens to which they had not previously responded, which was not performed previously, despite living with high levels of antigen (virus), because of their infection. This underscores an observation that will be discussed below – that different gene delivery systems (whether natural infection, DNA plasmid or other viral vector) result in different immune responses. This provides encouragement for the eventual success of developing vaccines against diseases such as HIV where natural infection – which had always been considered the gold standard for any vaccine – may not routinely induce immune responses adequate to clear the infection or provide protection against subsequent infection with a different strain. These results also set the stage for additional clinical trials for HIV and malaria where the DNA portion is intended to be the first component followed by a viral vector such as adenovirus or poxvirus encoding the same antigen genes.

**Table 2** Findings of DNA vaccine clinical trials

| |
|---|
| Well-tolerated safe |
| No integration of DNA |
| No autoimmunity |
| No tolerance |
| Antibody responses |
| Even in HIV-infected patients who did not make specific antibody with HIV infection (cytolytic T lymphocytes) CTL responses |
| In naive patients |
| Even in HIV-infected patients who did not make specific CTL with HIV infection |
| Th (helper T cells) responses |

## Second generation DNA vaccines

A variety of approaches are under evaluation to increase the potency of DNA vaccines (see Fig. 5) whilst still retaining their attractive features. Some of these are based upon devices to increase the transfection of cells or to target the DNA to specific sites, whilst also providing a means to avoid the traditional syringe (to facilitate global administration). These include propulsion devices targeting either the mucosa or benefiting from the transfection of Langerhans' cells in the skin. A mucosal jet injector device has been utilized in a clinical trial of an HIV DNA vaccine [26, 27]. The advantage of targeting the mucosa is that most pathogens enter the body via the mucosa, so that a vaccine administered

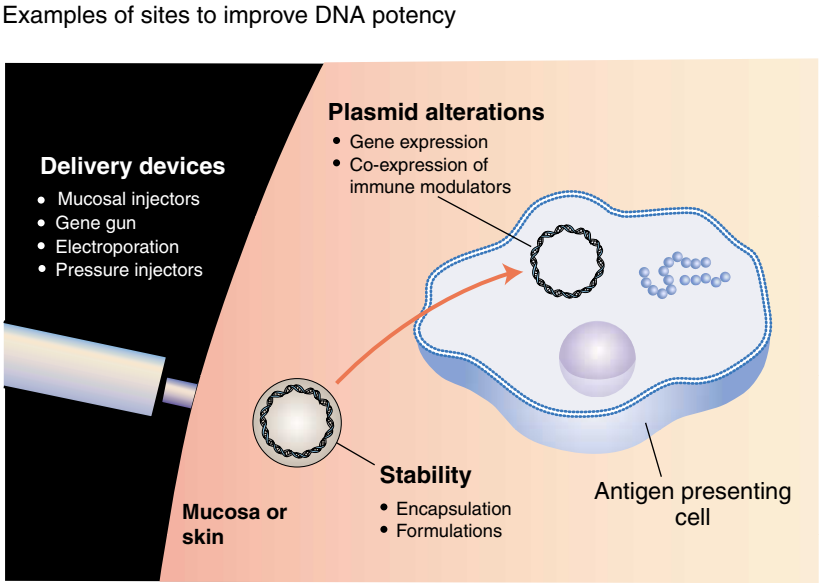


**Fig. 5** Second generation DNA vaccines. DNA vaccines with increased potency that are under development include vaccine delivery devices that inject the vaccine into the mucosa or epidermis without the use of needles. Alternatively, the uptake of the DNA into cells can be increased by the addition of small bursts of electric current by a process known as electroporation. The DNA can be encapsulated into microparticles to protect the DNA from degradation and to facilitate the uptake of the DNA by antigen presenting cells. The cellular mechanisms for transfection, DNA expression and antigen processing also provide targets for increasing potency.

mucosally may generate better mucosal (versus only systemic) immune responses. Electroporation devices are being evaluated that greatly increase the uptake of DNA into cells and expression of encoded protein [28, 29] by delivering small amounts of electric current *in vivo* to briefly cause the formation of hole in cells locally in order to permit more of the injected DNA to enter the cells.

Encapsulating DNA inside or onto entities such as microparticles [30, 31], or into bacteria [32, 33] is another means of either protecting the DNA from degradation and/or enhancing its uptake into antigen-presenting cells. Adjuvants such as aluminium salts likewise have been shown to increase the potency of the DNA vaccines [34]. Interestingly, the DNA itself has been shown to play a role in the immunogenicity of DNA vaccines [35–37]. This is because the bacterial DNA sequences result in the plasmid having a different methylation pattern than mammalian DNA. These sequences then activate the innate immune system, resulting in an augmentation of the antigen-specific immunity than would occur otherwise. However, to date, it is not yet known exactly as to how to manipulate the backbone sequences of the plasmid to fully exploit these observations. Addition of genes encoding cytokines or co-stimulatory molecules [38, 39] is also a promising means to augment the potency of immune responses or increase the protection observed in preclinical challenge models, or to alter the profile of the immune responses (such as the type of T-cell help).

## Mixed modality vaccines

A very promising strategy that is entering clinical trials is to combine DNA vaccines with other gene-delivery systems. Interestingly, it has been observed in a variety of preclinical systems that if DNA encoding an antigen is given as a prime, followed by another gene-based vector system (such as a recombinant poxvirus or adenovirus) encoding the same antigen, the immune responses and protection are significantly greater than if either vector is utilized for both the prime and boost, or if the order of administration is reversed [40–42]. Whilst the mechanisms for this increased potency remain to be established, the approach is being applied for HIV and malaria vaccines in clinical trials.

## Other applications of DNA vaccines

The DNA vaccines, or simply plasmids, have also found utility as a research tool. For example, whilst the field of genomics has revolutionized science with the elucidation of whole genomes of pathogens and living beings, one of the limitations has been to translate the sequence information into functional knowledge. Knocking out specific genes in mice strains is certainly a useful approach, but cumber-

some. Expressing the genes as proteins *in vivo* or *in vitro* can be carried out with viral vector delivery systems, but again, these are relatively time-consuming to make. Plasmid DNA can easily be utilized *in vitro* or *in vivo*. For pathogen genes, it is possible to develop DNA vaccine libraries [43] and use them to determine which genes encode protective antigens without even knowing what the gene encodes or the function of its corresponding protein. DNA vaccines have also been utilized to make polyclonal and monoclonal antibodies. This has enabled antibody production as a reagent without the need to purify the antigen, or to recombinantly produce and then purify the antigen in order to immunize for developing the antibodies.

## Conclusion

The DNA vaccines thus, in the decade since the initial demonstration of their efficacy, have rapidly advanced in clinical trials, with second generation formulations, delivery devices, and mixed modality approaches holding great promise for new vaccines and immunotherapeutics. At the same time, they are being utilized as research tools to help mine the vast amount of genetic information that has arisen from the field of genomics. The hope is that the fundamental simplicity of DNA vaccines combined with the sophisticated understanding of immune mechanisms and molecular biological manipulations will result in a platform technology useful for a variety of diseases (Table 3).

**Table 3** Applications of DNA vaccines under development

| |
|---|
| **Clinical applications** |
| Infectious diseases |
| Vaccines |
| Therapy |
| Cancer |
| Vaccines |
| Therapy |
| Autoimmune diseases |
| Allergy |
| **Technology toolbox** |
| Functional genornics |
| Antigen identification |
| Proteomics |
| Reagent generation |
| Polyclonal antibodies |
| Monoclonal antibodies |

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