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Enhancing DNA Vaccine Potency by Modifying the Properties of Antigen-Presenting Cells

Shaw-Wei D Tsen¹, Augustine Paik¹, Chien-Fu Hung^{1,2}, and T-C Wu^{1,4,*}

¹Department of Pathology, Johns Hopkins School of Medicine, Baltimore, MD USA

²Oncology, Johns Hopkins School of Medicine, Baltimore, MD USA

³Molecular Microbiology and Immunology, Johns Hopkins School of Medicine, Baltimore, MD USA

⁴Obstetrics and Gynecology, Johns Hopkins School of Medicine, Baltimore, MD USA

Abstract

DNA vaccines represent a potentially promising approach for antigen-specific immunotherapy. Advances in our knowledge of the adaptive immune system have indicated that professional antigen presenting cells (APCs), especially dendritic cells (DCs), play a key role in the generation of antigen-specific immune responses. Thus, the modification of the properties of DCs represents an important strategy for enhancing the potency of DNA vaccines. In the current review, we will discuss strategies to increase the number of antigen-expressing DCs, strategies to enhance antigen expression, processing, and presentation in DCs, strategies to promote the activation and function of DCs, and strategies to improve DC and T cell interaction, in order to optimize DNA vaccine-elicited immune responses. Continuing progress in our understanding of DC and T cell biology serves as a foundation for further improvement of DNA vaccine potency, which may lead to future clinical application of DNA vaccines for the control of infectious diseases and malignancies.

Keywords

DNA vaccines; antigen presenting cells; dendritic cells

2.0 INTRODUCTION

DNA vaccines have become an attractive and potentially effective strategy for antigen-specific immunotherapy. Naked DNA is relatively safe, stable, and easy to produce, and can be used to sustain high levels of antigen expression in cells (for a review, see [1,2]). In addition, since DNA vaccines do not elicit antibodies against DNA in the patient, they can be repeatedly administered. DNA vaccines, however, are poorly immunogenic because DNA lacks cell type specificity. Furthermore, DNA lacks the intrinsic ability to amplify or spread to surrounding cells *in vivo*. The potency of DNA vaccines can be enhanced by targeting DNA or encoded antigen to professional antigen presenting cells (APCs) and by modifying the properties of antigen-expressing APCs in order to boost vaccine-elicited immune responses.

Professional APCs, especially dendritic cells (DCs), are the central players in the initiation of the adaptive immune response. Accumulating evidence suggests that cell-mediated

* Address correspondence to: Department of Pathology, Johns Hopkins School of Medicine, Cancer Research Building II Room 309, 1550 Orleans St, Baltimore, MD 21231. Tel: (410) 614-3899; Fax: (443) 287-4295; wutc@jhmi.edu.

immunity as well as humoral immunity are critical for the control of viral infections and malignant tumors. It is now clear that DCs play a key role in the generation of antigen-specific antiviral and antitumor T cell immune responses. The generation of CD8⁺ T cells allows for direct killing of viral-infected cells or tumors, while the generation of CD4⁺ T helper cells leads to the augmentation of both CD8⁺ immune responses as well as antibody-mediated immunity. DCs are chiefly responsible for the presentation of antigen to naïve CD4⁺ and CD8⁺ T cells and for the activation of armed effector CD4⁺ and CD8⁺ T cells. Immature DCs, which are located in peripheral tissues, efficiently uptake antigens, process the antigens into antigenic peptides, and load the peptides onto major histocompatibility (MHC) class I and class II molecules in order to present them on the cell surface. These immature DCs possess various types of surface receptors, including toll-like receptors (TLRs), cytokine receptors, FcR, tumor necrosis factor (TNF)-receptor family molecules, and sensors for cell death (for a review, see [3]), which enable them to respond to danger signals, such as bacterial or viral components or inflammatory cytokines indicating the presence of an infection. In response to a danger signal, the DCs undergo a maturation process, upregulating adhesion and co-stimulatory molecules and transforming into efficient APCs and potent activators of T cells. These DCs then migrate to the lymphoid organs to select and stimulate antigen-specific T cells (for a review, see [3-5]).

Therefore, strategies to enhance the potency of DNA vaccines should focus on: 1) increasing the number of antigen-expressing DCs; 2) improving antigen expression, processing, and presentation in DCs; 3) promoting DC activation and function; and 4) enhancing DC and T cell interaction, to augment vaccine-elicited T cell immune responses.

3.0 Strategies to increase the number of antigen-expressing and/or antigen-loaded DCs

3.1 Intradermal administration of DNA vaccines via gene gun and intranodal injection as efficient routes for the delivery of DNA to DCs

One strategy for increasing antigen-expressing DC populations is to find convenient and effective routes for the delivery of DNA vaccines directly into DCs *in vivo*. To date, DNA vaccines have been most commonly administered via either the intramuscular or intradermal route (for a review, see [2]). Among the previously explored routes of DNA administration, intradermal vaccination via gene gun represents one of the most efficient methods for delivering DNA directly into DCs. The gene gun can deliver DNA-coated gold particles into intradermal Langerhans cells *in vivo* [6]; these cells then mature and migrate to the lymphoid organs for T cell priming [7]. In addition, gene gun immunization is significantly more dose-efficient than intramuscular or subcutaneous injection (for reviews, see [2,8]).

Another important method for direct delivery of DNA to DCs is via intranodal injection of DNA vaccines. Several groups have shown that intralymphatic delivery of DNA vaccines into the lymph nodes or spleen can also significantly enhance the immunogenicity of DNA vaccines [9-11]. By targeting DNA encoding antigen to DC-rich areas such as the secondary lymphoid tissues, intralymphatic immunization may increase the likelihood of transfecting large numbers of local APCs and result in strong CD8⁺ T cell immune responses [9].

3.2 Electroporation as an efficient method for improving cellular DNA uptake to increase the number of antigen-expressing DCs

Once DNA vaccines have been delivered, however, their potency may be limited by inefficient cellular DNA uptake *in situ*. The employment of electroporation, a technique widely used to improve *in vitro* gene transfection, can potentially facilitate the uptake and expression of DNA by target cells *in vivo* and lead to an increase in the number of antigen-

expressing DCs, augmenting vaccine-elicited immune responses. Several reports have demonstrated improved antigen expression and enhanced DNA vaccine-elicited antigen-specific immune responses by electroporation *in vivo* [12-18]. Thus, electroporation may represent a strategy for overcoming the barrier of limited DNA transfection efficiency after the delivery of DNA vaccines.

3.3 Intercellular antigen spreading as a strategy to increase the number of antigen-expressing DCs

The potency of DNA vaccines is still limited by the inability of naked DNA to naturally amplify and spread among DCs *in vivo*. This limitation can potentially be overcome by promoting the spread of encoded antigen from transfected cells to DCs. Linkage of antigen to proteins capable of intercellular transport has been shown to facilitate the spread of encoded antigen. For example, the herpes simplex virus type 1 (HSV-1) tegument protein VP22, when linked to thymidine kinase [19], cytosine deaminase [20], p53 [21], and green fluorescent protein (GFP) [22], has been shown to distribute the protein to the nuclei of surrounding cells. A fusion gene consisting of VP22 linked to a model antigen, human papillomavirus type 16 (HPV-16) E7, was demonstrated to dramatically increase the number of E7-expressing DCs in the lymph nodes of mice [23] as well as enhance E7-specific long-term memory CD8⁺ T cell immune responses and antitumor effects against E7-expressing tumor cells [23,24]. Similarly, HSV-1 VP22 has been employed in a DNA vaccine to enhance vaccine-elicited immune responses against HPV-16 E6 [25] as well as influenza NP antigen [26].

Two other proteins with some homology to VP22, bovine herpesvirus VP22 (BVP22) and Marek's disease virus VP22 (MVP22), appear to be capable of intercellular spreading and transport as well [27-30]. Immunization of mice with a DNA vaccine encoding BVP22 linked to yellow fluorescent protein (YFP) was shown to generate greater CD8⁺ T cell immunity against YFP than administration with YFP DNA alone [31]. In addition, administration of a DNA vaccine encoding BVP22 linked to bovine herpesvirus-1 glycoprotein D (tgD) was shown to generate stronger tgD-specific immune responses in mice [32] as well as in cattle [33] compared to vaccination with DNA encoding tgD alone. Similarly, vaccination with a fusion gene consisting of MVP22 linked to HPV-16 E7 was shown to enhance E7-specific CD8⁺ T cell responses and antitumor effects in mice relative to immunization with wild-type E7 DNA [34].

The concern has been raised that the intercellular trafficking ability of VP22 may potentially be attributed to fixation artifacts [35]. In addition, one group found that transfection of mammalian cells with DNA encoding HSV-1 VP22 fused to enhanced GFP (EGFP) did not lead to increased intercellular spread of the fusion protein *in vitro*, but could lead to significantly enhanced production of EGFP-specific antibodies in mice vaccinated with this construct [36]. However, the transport properties of VP22 without fixation have been demonstrated [31]. In particular, binding and transport of a RNA (U_s8.5-EGFP) into surrounding cells by VP22 without fixation has been shown [37]. Since no intercellular transport of U_s8.5-EGFP mRNA was observed in the absence of VP22, this study supports the conclusion that VP22 can move between cells. To date, the ability of VP22 to bind and transport non-U_s8.5-EGFP mRNA has not been shown. However, if these issues are addressed, it is possible that in the future co-administration of DNA encoding VP22 and DNA encoding antigen of interest may represent a method for the distribution of antigen to surrounding cells.

3.4 Linkage of antigen to molecules capable of binding to DCs as a method to target antigen to DCs

Another strategy to increase the number of antigen-expressing or antigen-loaded DCs is the linkage of antigen to molecules that target the antigen to the surface of DCs in the context of DNA vaccines. For example, DNA vaccines encoding antigen linked to a secreted form of heat shock protein 70, which bind to scavenger receptors on the surface of DCs such as CD91, may represent an effective method for targeting linked antigen to DCs and enhancing antigen-specific immunity [38-40]. Linkage of antigen to Fms-like tyrosine kinase 3-ligand can also target antigen to DCs; a chimeric Flt-3L-E7 fusion gene was shown to significantly improve cytotoxic T lymphocyte (CTL) responses in mice compared to vaccination with wild-type E7 DNA [41]. Linkage of antigen to cytotoxic lymphocyte antigen 4 (CTLA-4), which binds to B7-expressing cells such as DCs, can also direct antigen to sites of immune induction and enhance the vaccine-generated immune response [42].

Furthermore, linkage of antigen to the Fc portion of immunoglobulin G (IgG) can target antigen to Fc receptors on the surface of DCs, promote receptor-mediated internalization of the antigen-Fc complexes by DCs and result in enhanced vaccine potency. For example, You *et al.* have demonstrated that a DNA vaccine encoding a model hepatitis B virus *e* antigen (HBeAg) linked to an IgG Fc fragment could elicit broad enhancement of antigen-specific CD4⁺ and CD8⁺ T cell and B cell immune responses in mice [43]. Thus, this strategy may be capable of targeting antigen to DCs as well as augmenting vaccine-elicited immune responses from multiple arms of the adaptive immunity.

3.5 Employment of chemotherapy-induced apoptotic cell death to increase the number of antigen-loaded DCs

In the presence of an established tumor, co-administration of DNA vaccines with chemotherapeutic agents that promote the release of antigen from apoptotic tumor cells can potentially facilitate antigen uptake by local DCs, resulting in enhancement of vaccine potency. For example, Kang *et al.* have recently shown that the chemotherapeutic agent epigallocatechin-3-Gallate (EGCG), a chemical derived from green tea, could induce tumor cellular apoptosis and enhance the tumor antigen-specific T cell immune responses elicited by DNA vaccination [44]. However, it is important to note that such a strategy is more appropriate in a therapeutic context (i.e. in the presence of a tumor) because in the absence of a tumor, EGCG actually leads to decreased DNA vaccine potency, presumably acting as an suppressor of DC function [44]. It may be of interest to explore whether other chemotherapeutic agents could exhibit similar synergistic effects when combined with DNA vaccines.

4.0 Strategies to improve antigen expression, processing, and presentation in DCs

4.1 Codon optimization as a strategy to enhance antigen expression in DCs

Codon optimization has emerged as an important strategy to enhance the expression of encoded antigen in DCs. This technique involves the modification of antigenic gene sequences via replacement of codons rarely recognized by cellular protein synthesis machinery with more commonly recognized codons, and can enhance the translation of DNA vaccines in DCs. Immunization of mice with either codon-optimized HPV-16 E6 DNA [45] or codon-optimized HPV-16 E7 DNA [46] was shown to generate greater immune responses than vaccination of mice with wild-type E6 or E7 DNA, respectively.

4.2 Employment of intracellular targeting strategies to enhance MHC class I and class II antigen presentation in DCs

Increased progress in our understanding of intracellular pathways for antigen presentation has created the opportunity to enhance DNA vaccine potency. Thus, strategies to facilitate MHC class I antigen processing in DCs can lead to the activation of larger populations of CD8⁺ T cells and the generation of stronger antitumor or antiviral immunity. It has been demonstrated that linkage of antigen to proteins that target the antigen for proteasomal degradation or entry into the endoplasmic reticulum (ER) can facilitate MHC class I presentation of linked antigen in DCs. For example, linkage of antigen to *Mycobacterium tuberculosis* heat shock protein 70 (hsp70) [47], γ -tubulin [48], calreticulin (CRT) [49], ER insertion signal sequences [50], ubiquitin [51], or the translocation domain of *Pseudomonas aeruginosa* exotoxin A (ETA(dII)) [52] can significantly improve MHC class I presentation of nuclear or cytosolic antigens (see Figure 1). In a head-to-head comparison of HPV-16 E7 DNA vaccines employing intracellular targeting strategies, Kim *et al.* found that a DNA vaccine encoding E7 linked to CRT generated the greatest E7-specific CTL responses and antitumor effects against E7-expressing tumors in a preclinical model [53]. The encouraging results from these preclinical studies have led to several proposed HPV DNA vaccine trials in humans (Wu, personal communications).

Strategies have also been developed to enhance MHC class II presentation of antigen encoded by DNA vaccines. It is increasingly evident that CD4⁺ helper T cells also play a major role in the priming of CD8⁺ T cells as well as the generation of memory T cells [54]. Thus, strategies to facilitate MHC class II presentation of vaccine-encoded antigen can significantly improve the potency of the DNA vaccines. For example, Wu *et al.* have demonstrated that fusion of HPV-16 E7 with the sorting signal of lysosomal-associated membrane protein type 1 (LAMP-1) can target the E7 antigen to cellular endosomal/lysosomal compartments and facilitate class II presentation of E7 [55]. This construct was tested in the context of a DNA vaccine and generated higher numbers of E7-specific CD4⁺ T cells as well as greater E7-specific CTL activity in mice than DNA vaccines encoding wild-type E7 alone [56]. In addition, a DNA vaccine encoding the human immunodeficiency virus-1 (HIV-1) Gag antigen linked to LAMP-1 was shown to elicit enhanced long-term antibody responses and CD4⁺ and CD8⁺ T cell memory responses compared to vaccination with wild-type Gag alone [57]. Others have shown that vaccination with DNA encoding antigen linked to melanosome transport sorting signals [58] or transferrin receptor [59] can also lead to enhanced MHC class II antigen presentation to CD4⁺ T cells relative to immunization with DNA encoding wild-type antigens alone.

A recently developed DNA vaccine strategy involves the employment of MHC class II-associated invariant chain (Ii) for improving class II presentation of antigen in DCs. In the ER, Ii binds with MHC class II molecules, and the class II-associated peptide (CLIP) region of Ii occupies the peptide-binding groove of the MHC molecule, preventing premature binding of antigenic peptides into the groove (for reviews, see [60,61]). In the endosomal/lysosomal compartments, CLIP is replaced by a peptide antigen, and the MHC class II molecule/antigenic peptide complex is then presented on the cell surface. By substituting the CLIP region of Ii with a desired epitope, the epitope can be efficiently presented through the MHC class II pathway in DCs for the stimulation of peptide-specific CD4⁺ T cells (see Figure 2). Previous studies have shown that transfection of MHC class II-positive cells with DNA encoding an Ii chain in which CLIP is replaced with a CD4⁺ T cell epitope of an antigen of interest can lead to the presentation CD4⁺ T cell epitope through the MHC class II pathway [62-64]. More recently, Hung *et al.* have demonstrated that a DNA vaccine encoding Ii with the CLIP region replaced with the pan HLA-DR binding epitope (PADRE) could elicit potent PADRE-specific CD4⁺ T cell responses in vaccinated mice [65]. In addition, a co-administration of this vaccine (termed Ii-PADRE) with DNA encoding

HPV-16 E7 generated significantly greater CD8⁺ T cell immune responses relative to a co-administration of DNA encoding HPV-16 E7 with DNA encoding unmodified Ii [65].

4.3 Bypassing antigen processing as a method for generating stable antigen presentation in DCs

Recent studies have demonstrated the possibility of circumventing antigen processing altogether for the generation of stable MHC class I presentation. The employment of MHC class I single-chain trimer (SCT) technology involves linkage of the gene encoding an antigenic peptide to β 2-microglobulin and a MHC class I heavy chain, producing a single-chain construct encoding the peptide antigen fused to an MHC class I molecule (see Figure 3) (for a review, see [66]). The expression of this construct within DCs may allow for stable presentation of the antigenic peptide through MHC class I molecules on the cell surface. For example, a DNA vaccine encoding a SCT encoding a HPV-16 E6 CTL epitope, β 2-microglobulin, and H-2K^b MHC class I heavy chain has been shown to generate enhanced E6 peptide-specific CD8⁺ T cell responses in vaccinated mice relative to immunization with DNA encoding wild-type HPV-16 E6 [67]. In addition, a DNA vaccine employing an SCT of human leukocyte antigen (HLA)-A2 linked to a peptide of the tumor-associated antigen mesothelin was recently shown to generate strong mesothelin peptide-specific CD8⁺ T cell responses and prevent the growth of mesothelin-expressing tumors in mice [68].

5.0 Strategies to promote DC activation and function

5.1 Employment of toll-like receptor ligands and other genetic adjuvants as a strategy to enhance DNA vaccine potency

DC activation is a prerequisite to T cell priming and the generation of antigen-specific immune responses. In the presence of “alert” signals such as toll-like receptor (TLR) ligands or inflammatory cytokines, DCs are stimulated to mature and differentiate into potent activators of antigen-specific T cells (for reviews, see [3]). The strength of the immune response elicited by DNA vaccines can thus be enhanced by strategies that provide “alert” signals and other maturation-inducing stimuli to DCs in order to facilitate DC activation. For example, CpG motifs, which are bacterial-derived immunostimulatory gene sequences, can promote the growth and activation of transfected DCs via stimulation through TLR-9 [69] and enhance DNA vaccine potency [70]. Specifically, since DNA vaccines are constructed from bacterial DNA plasmids, CpG motifs contribute significantly to the intrinsic immunogenicity of DNA vaccines [71]. In addition, several groups have shown that modification of the CpG content of a DNA plasmid backbone can affect the immunogenicity of the plasmid [72,73]. Furthermore, co-administration of DNA vaccines with CpG-enriched DNA plasmids can also enhance DNA vaccine potency [74].

Other TLR ligands may also be useful for enhancing DNA vaccine potency through their actions on TLRs on the surface of DCs. It is now clear that TLRs on DCs play a crucial role in innate and adaptive immune responses (for reviews, see [75-77]). Thus, DNA vaccines administered in conjunction with agents that modify TLR-mediated signal transduction pathways may lead to DC activation, resulting in enhanced vaccine-elicited immune responses. For example, imiquimod and its analog resiquimod are two immune response modifiers that have been shown to bind to and activate TLR-7 and/or TLR-8 on DCs [78,79]. Administration of imiquimod and resiquimod as DNA vaccine adjuvants led to an increase in the number and maturation status of DCs in lymphoid organs [80] and was shown to enhance tumor-specific [81] and viral antigen-specific [82,83] immune responses generated by DNA vaccines in mice.

TLR-4 ligands have also been shown to enhance DNA vaccine potency. For example, monophosphoryl lipid A (MPL), a detoxified derivative of the lipopolysaccharide (LPS) of

Salmonella minnesota R595 [84], has been shown to activate DCs via TLR-4 (for a review, see [85]). Sasaki *et al.* have shown that administration of MPL in conjunction with DNA vaccines could enhance both antibody- and CTL-mediated immunity induced by DNA vaccination against HIV-1 viral-encoded antigens in mice [86].

In addition to the employment of TLR ligands, the use of DNA encoding TLR adaptor molecules can also improve the potency of DNA vaccines. Takeshita *et al.* recently found that DNA vaccination in conjunction with DNA encoding the TLR adaptor molecules myeloid differentiation factor 88 (MyD88) and Toll-IL-1 receptor domain-containing adaptor-inducing IFN- β (TRIF) could enhance the antigen-specific immune responses generated by DNA vaccines [87]. These findings suggest that DNA encoding TLR adaptor molecules may be useful as genetic adjuvants for augmenting DNA vaccine-elicited immune responses via enhanced DC activation.

Other strategies that have been used to activate DCs include the employment of cationic lipid-DNA complexes (CLDCs) consisting of liposomes complexed to plasmid DNA [88]. When systemically delivered as a vaccine, CLDCs were shown to induce the release of significant levels of T helper type 1 (Th1)-biasing cytokines such as IL-12 by DCs, leading to enhanced antigen-specific immune responses [89]. In addition, it was demonstrated that CLDCs were selectively taken up by splenic DCs, and thus CLDCs may represent a method for the efficient transfection of DCs *in vivo* as well as for the activation of DCs [88,89].

Other immunostimulatory adjuvants for DNA vaccination which may be related to DC activation include cholera toxin (CT) [90] and *Escherichia coli* heat-labile enterotoxin (LT) [91,92], two closely related molecules capable of inducing maturation in DCs and eliciting robust immune responses when administered via the mucosal and parenteral routes (for reviews, see [93-95]). For example, Arrington *et al.* have shown that intradermal administration of DNA vaccines in conjunction with CT and LT plasmid vectors could significantly enhance Th1 and Th2 cytokine responses against viral antigens and induce strong protective immunity [96]. Thus, these and other bacterial toxin-based adjuvants may represent effective means of promoting the activation of DCs to enhance DNA vaccine potency.

5.2 Inhibition of immunosuppressive factors to enhance DC function

Another relatively novel and interesting approach to enhancing DC activation is the inhibition of natural immune attenuators in DCs. Such immunoinhibitory molecules include suppressor of cytokine signaling 1 (SOCS-1), which participates in the negative regulation of the Jak-STAT pathway and suppresses DC activation [97] (for a review, see [98]). Song *et al.* have recently shown that a co-administration of HIV-1 DNA vaccines with DNA encoding small interfering RNA (siRNA) targeting SOCS-1 could significantly improve DNA vaccine-elicited HIV-1 envelope-specific CD8⁺ and CD4⁺ T cell immune responses, presumably via enhanced production of inflammatory cytokines such as interleukin (IL)-12 by DCs [99]. These studies suggest that in the future, inhibition of other immunosuppressive factors in DCs may also represent effective strategies for augmenting DNA vaccine potency.

6.0 Strategies to enhance DC and T cell interaction

6.1 Employment of cytokines and co-stimulatory molecules to enhance T cell activation

The stimulation of antigen-specific T cells by DCs during T cell priming is dependent on two signals delivered by DCs to T cells. First, the appropriate peptide-MHC complexes on the surface of DCs must interact with T cell receptors. Second, co-stimulatory molecules on the surface of DCs must stimulate their counterparts on T cells. Cytokines secreted by DCs may augment or even serve as the secondary co-stimulatory signal. Studies have shown that

co-delivery of DNA vaccines with DNA encoding various cytokines such as IL-2, IL-12, IL-18, or granulocyte-macrophage colony stimulating factor (GM-CSF) can enhance vaccine-elicited immune responses (for a review, see [2,100,101]). Furthermore, co-administration of DNA vaccines with DNA encoding cytokines fused to Fc domains of IgG has been shown to further enhance DNA vaccine potency [102]. Co-administration of DNA vaccines with DNA encoding co-stimulatory molecules such as B7 may also lead to more efficient activation of antigen-specific T cells [7].

6.2 Prolonging DC survival to enhance T cell interaction

After T cell priming, DCs may become targets of T cell-mediated apoptosis. Thus, strategies to prolong the life of DCs may further increase the number of activated T cells. Kim *et al.* have developed a strategy to prolong DC survival using DNA encoding antiapoptotic proteins, including Bcl-xL, Bcl-2, X-linked inhibitor of apoptosis protein (XIAP), and dominant negative mutants (dn) of caspases such as dn caspase-9 and dn caspase-8. This group showed in mice that co-administration of a DNA vaccine encoding HPV-16 E7 with DNA encoding any of these antiapoptotic factors could augment E7-specific CTL responses and elicit potent antitumor effects against E7-expressing tumors [103]. However, delivery of DNA encoding antiapoptotic proteins into cells raises concerns for cellular malignant transformation. The use of gene knockdown strategies such as RNA interference (RNAi) targeting key proapoptotic proteins may provide a novel method for accomplish the same goal while alleviating the concern of oncogenicity. Kim *et al.* have also shown that co-administration of a DNA vaccine encoding HPV-16 E7 with siRNA targeting the key proapoptotic proteins Bak and Bax can prolong the survival of transfected DCs and augment E7-specific CD8⁺ T cell responses against E7-expressing tumors in mice [104]. These studies suggest that prolongation of DC survival may represent an effective strategy for improving antigen presentation by DCs to T cells and for potentiating vaccine-elicited T cell immune responses. It would be of interest to further explore co-administration of DNA vaccines with siRNA targeting other key proapoptotic proteins, such as caspase-8, caspase-9 and/or caspase-3, to enhance DNA vaccine potency.

6.3 Induction of CD4⁺ T cell help as a strategy for augmenting CD8⁺ T cell responses

The activation of CD8⁺ T cells is dependent upon, and augmented by, T cell help from CD4⁺ T cells. Thus, strategies to induce CD4⁺ T helper cells at sites of CD8⁺ T cell priming can potentially enhance CTL immune responses. For example, Hung *et al.* have shown that co-administration of DNA vaccines with DNA encoding an Ii chain with the CLIP region replaced with pan HLA-DR binding epitope (PADRE) (see Section 4.2.) could lead to significant enhancement in antigen-specific CD8⁺ immune responses [65]. Furthermore, it has been shown that DNA vaccines encoding fragment C (FrC) of tetanus toxin linked to an antigenic peptide could significantly enhance antigen-specific CD8⁺ T cell immune responses [105-107]. The FrC of tetanus toxin contains universally immunogenic T helper cell epitopes which can bind to many different types of MHC class II molecules [108]. Thus, the observed enhancements in antigen-specific CD8⁺ T cell responses generated by DNA encoding the fusion protein are likely due to CD4⁺ T cell help created by FrC of tetanus toxin. This suggests a potentially effective vaccine strategy for CD4⁺ T cell-mediated augmentation of CD8⁺ T cell priming for potentiating DNA vaccines.

6.4 Eliminating immunosuppressive regulatory T cells as a method to improve T cell priming

It has now become clear that the presence of natural regulatory T cells (T_{reg} cells) can limit the magnitude of effector immune responses against infection and malignancies. T_{reg} cells, which are normally important for the preservation of host homeostasis, constitutively express CD25 and the T cell inhibitory receptor CTLA-4 and directly and indirectly

participate in the inhibition of DC and CD8⁺ T cell interaction (for reviews, see [109,110]). Thus, the depletion of T_{reg} cells is a potential strategy for enhancing the potency of DNA vaccines. Several agents have been used to deplete T_{reg} cells *in vivo*, such as cyclophosphamide, fludarabine, IL-2 immunotoxin, and COX-2 inhibitors (for a review, see [110]). Administration of DNA vaccines in conjunction with agents to deplete T_{reg} cells has been shown to result in enhancement in antigen-specific CD8⁺ T cell responses [111,112]. In this regard, strategies to deplete T_{reg} cells may represent an effective means of eliminating negative immunoregulation and enhancing T cell activation and function, potentiating the effects of DNA vaccines.

6.5 Promoting *in vivo* DC expansion to improve T cell activation

Another strategy to improve the interactions between DCs and T cells is the employment of cytokines involved in DC expansion, such as the potent DC growth factor Fms-like tyrosine kinase 3-ligand (Flt-3L). Co-administration of a DNA vaccine encoding antigen with DNA encoding Flt-3L has been shown to recruit large numbers of DCs and generate significant antigen-specific immune responses in mice [113]. Furthermore, it was recently shown that a DNA vaccine encoding a fusion protein comprising the tumor antigen MUC-1 and human Flt-3L could elicit MUC-1-specific CTL responses and potent tumor regression in mice [114]. As mentioned above, linkage of antigen to Fms-like tyrosine kinase 3-ligand may also target antigen to DCs, resulting in improved cytotoxic T lymphocyte (CTL) responses in mice [41] (see Section 3.4).

Strategies to expand DCs *in vivo* can be coupled with strategies to attract DCs to sites of DNA vaccination to further enhance vaccine potency. For example, Sumida *et al.* have shown that co-administration of plasmids encoding the chemokine macrophage inflammatory protein-1alpha (MIP-1alpha) and Flt-3L with a DNA vaccine could result in enhanced recruitment, expansion, and activation of DCs at the site of DNA vaccination and lead to augmented antigen-specific cellular and humoral immune responses elicited by the vaccine [115].

7.0 EXPERT COMMENTARY AND FIVE-YEAR VIEW

DCs play a critical role in the generation of CD4⁺ and CD8⁺ T cell immune responses against viruses and malignancies. Thus, it is logical to explore strategies to enhance the function and survival of DCs in order to improve DNA vaccine-elicited T cell immune responses. We expect further advancement in the DNA vaccine strategies discussed in the current review related to 1) increasing the number of antigen-expressing DCs, 2) improving antigen expression, processing, and presentation in DCs, 3) promoting the activation and function of DCs, and 4) enhancing DC and T cell interaction. In the future, combinations of these approaches will likely yield further improvements in the potency of DNA vaccines. Furthermore, continued advancement in our understanding of fundamental immunology will be useful for the generation of novel strategies for enhancing the potency of DNA vaccines.

Ultimately, for the control of disease it is important to seek other strategies to further enhance DNA vaccine potency due to the low immunogenicity of DNA vaccines. Additional strategies to improve DNA vaccine potency include the employment of prime-boost strategies. For example, priming with DNA vaccines and boosting with vaccinia has been shown to be an effective method to further enhance DNA vaccine potency (for a review, see [116]). In addition, combination of DNA vaccination with immunostimulatory adjuvants, such as TLR ligands (see Section 5.1) and/or chemotherapeutic agents such as EGCG (see Section 3.5) can also potentiate DNA vaccines. Furthermore, elimination of immunosuppressive factors such as regulatory T cells can also enhance DNA vaccine-elicited immune responses (see Section 6.4).

Impressive results from preclinical models have led to several ongoing clinical trials with DNA vaccines against infectious diseases, including human immunodeficiency virus (HIV) [117,118], hepatitis B virus (HBV), dengue, influenza, and malaria [119-121] [122]. In addition, DNA vaccines have also been employed to control cancers such as human papillomavirus (HPV)-associated cervical cancer [123,124], prostate cancer [125], melanoma, renal-cell carcinoma, breast and ovarian cancers, and non-small cell lung carcinomas (for a review, see [116], see also www.clinicaltrials.gov). These clinical studies allow us to identify the relevant characteristics and mechanisms of the immune response that lead to the greatest enhancements in DNA vaccine potency. Such immunological parameters will be useful for characterizing effective immune mechanisms in the development of better DNA vaccines.

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Key issues

- Naked DNA as an important form of vaccines.
- Dendritic cells as a central player for DNA vaccine development.
- Increasing number of antigen expressing dendritic cells to enhance DNA vaccine potency.
- Improving antigen expression, processing, and presentation in DCs to enhance DNA vaccine potency.
- Promoting DC activation and function to DNA vaccine potency.
- Augmenting DC and T cell interaction to improve DNA vaccine potency.

Summary

DNA vaccines have become an attractive approach for antigen-specific immunotherapy. Continued progress in our understanding of adaptive immunity has indicated that antigen-presenting cells, in particular dendritic cells, play a crucial role in the generation of antiviral and antitumor immune responses. Therefore, modification of the properties of antigen-presenting cells provides an innovative strategy for enhancing the potency of DNA vaccines. In addition, it is expected that future advancements in our knowledge of fundamental immunology will help pave the way for the development of more effective DNA vaccines.

Figure 1.

Antigen presentation pathways in DCs. In the MHC class I presentation pathway, cytosolic antigens are targeted for proteasomal degradation. The resulting antigenic peptides associate with MHC class I molecules in the ER and are transported to the cell surface. In the MHC class II pathway, exogenous antigens are endocytosed and the vesicle containing antigen is fused with lysosomes, facilitating antigen degradation. The resulting vesicle is then fused with a vesicle containing MHC class II molecules, and the MHC class II molecule/antigenic peptide complexes are transported to the surface of the cell. In the context of DNA vaccines, linkage of antigen to hsp, CRT, or ETA(dII) targets antigen for proteasomal degradation and/or entry into the ER, enhancing class I presentation of antigen. Linkage of antigen to LAMP-1, Ii, or TfR targets antigen to endosomal/lysosomal compartments for improved class II antigen presentation. DC, dendritic cell; MHC, major histocompatibility complex; ER, endoplasmic reticulum; hsp, heat shock proteins; CRT, calreticulin; ETA(dII), translocation domain of *Pseudomonas aeruginosa* exotoxin A; LAMP-1, lysosomal-associated membrane protein type 1; Ii, MHC class II-associated invariant chain; TfR, transferrin receptor.

Figure 2.

Employment of Ii for stable presentation of a desired antigenic peptide by DCs. The translated Ii-antigen peptide enters the ER and binds to an MHC class II molecule, with the antigenic peptide occupying the peptide-binding groove of the MHC molecule. In the lysosomal compartments, the Ii chain is degraded, leaving the desired antigenic peptide in the peptide-binding groove of the MHC class II molecule and allowing for the stable presentation of the antigenic peptide on the cell surface.

Figure 3.
 (a) Illustration of a SCT (comprising antigenic peptide, β_2 -microglobulin, and MHC class I heavy chain) on the cell surface. (b) The SCT gene construct.