Journal of Cellular Biochemistry 98:235–242 (2006)

PROSPECTS

DNA Vaccines: Successes and Limitations in Cancer and Infectious Disease

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Abstract Vaccination with plasmid DNA is an active area of investigation that is being applied to diseases including cancer and microbial pathogens associated with infectious diseases. Since its discovery, great progress has been made with the administration of DNA vaccines to initiate specific and effective immune responses against targeted illnesses. However, many obstacles still face its use in prophylactic and therapeutic vaccination scenarios. The nature of these difficulties alongside the successes and future of plasmid DNA will be discussed. J. Cell. Biochem. 98: 235–242, 2006.

Key words: DNA vaccines; cancer; immunotherapy; infectious diseases

The ease in production and minimal safety concerns of plasmid DNA, genetically engineered to encode an antigen or transgene of interest, has rendered it great potential for use in prophylactic and therapeutic vaccination scenarios involving human disease. The ability of these DNA vectors to also activate both arms of the immune system (i.e., cellular and humoral) against an encoded gene product has resulted in its intensive study for vaccine development and as an immunotherapeutic modality. Therefore, the scope of this review serves to bring to light the advancements and challenges facing DNA vaccines, particularly in protection against human cancer and infectious disease.

HISTORICAL PERSPECTIVES

The application of plasmid DNA as an immunogenic delivery moiety for foreign gene products is a relatively recent observation. In the early 1990s, a number of animal model studies first indicated success in the delivery system and protein expression of vaccinating with DNA preparations [Wolff et al., 1990; Williams et al., 1991]. With time, these results and others clearly demonstrated the ability of DNA vaccines to activate many components of the immune system, including B and T lymphocytes, in rodents and non-human primates specifically to influenza [Robinson et al., 1993; Ulmer et al., 1994], human immunodeficiency virus type-1 (HIV-1) [Wang et al., 1993], and a number of cancer modalities. DNA vaccination was found to have many potential advantages over other vaccine strategies, and the successes in these early animal studies and the advancement of plasmid DNA technology led to the first human clinical trial monitoring the safety and response of DNA vaccination against HIV-1 infection [MacGregor et al., 1998].

Abbreviations used: HIV-1, human immunodeficiency virus type 1; MHC, major histocompatibility class; APC, antigen presenting cell; MALT, mucosal-associated lymphoid tissues; NK, natural killer; Th, T helper; IL, interleukin; IFN-γ, interferon gamma; i.v., intravenous; PSA, prostate-specific antigen; SV40, Simian virus 40; Tag, large tumor antigen; MPM, malignant pleural mesothelioma; CMV, cytomegalovirus; JE, Japanese encephalitis; DEN, dengue; WN, West Nile; JE-VAX, JE vaccine; CTL, cytotoxic T lymphocyte.

Grant sponsor: National Institutes of Health; Grant number: RR-12317.

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Received 16 November 2005; Accepted 17 November 2005
DOI 10.1002/jcb.20775

DIFFICULTIES ASSOCIATED WITH DNA VACCINES TARGETING CANCER

The efficacy of DNA vaccines has been observed most successfully in murine settings involving cancer by targeting tumor-associated or tumor-specific antigens [reviewed in Weiner and Kennedy, 1999]. In contrast, administration within human systems [Conry et al., 2002; Rosenberg et al., 2003; Triozzi et al., 2005] has achieved little success due to the inability of DNA vaccines to elicit robust immune responses during the course of early clinical trials. The obstacles confronting the inefficiencies of DNA vaccines are several-fold. First, the oncogenic process is normally the result of tissue outgrowth with self-antigen over-expression, and breaking tolerance against constitutive tumor-associated antigen targets remains troublesome from the prospect of generating autoimmune reactions. Difficulties also arise from choosing an appropriate vaccine antigen based upon upregulation of expression in normal versus abnormal tissues. Second, the tumor microenvironment might be conducive to shielding itself from presentation to the immune system through immunosuppressive cytokine secretion [Gorelik and Flavell, 2001; Yang and Lattime, 2003] or activation of CD4+ CD25+ T-regulatory cells exhibiting a suppressor phenotype [Shimizu et al., 1999]. Lastly, tumor evasion could be a result of immunoediting [reviewed in Dunn et al., 2002] in which disease occurs from the selection and proliferation of tumor cells with downregulated surface molecules, unable to elicit an appropriate immune reaction. Indeed, neoplasms within murine models have been observed to express decreased levels of costimulatory molecules [Singh et al., 2003] and the machinery necessary for effective antigen presentation [Garcia-Lora et al., 2003]. Similarly, many human cancers have shown loss of proper presentation in human leukocyte antigens [Kloor et al., 2005; Riemersma et al., 2005; So et al., 2005], costimulatory molecules [Stopeck et al., 2000], and tumor-associated antigens [Kontani et al., 2001; Khong et al., 2004] with the progression of disease, indicating the potential effects of immune selection.

DNA vaccines hold considerable promise as a useful tool to prevent tumorigenic growth and subsequent metastasis. Unfortunately, the anticipated success of DNA vaccines to promote systemic immunity in humans has been disappointing. The vaccine is often employed therapeutically and as a last resort option with immunocompromised patients during late stage disease after standard treatments of radiation, chemotherapy, and surgery. Rather then abandoning this form of vaccination, additional tactics must be employed in order to achieve an optimum immune response to initiate long-lived immunity.

APPROACHES TO VACCINATING WITH PLASMID DNA

Several strategies exist in order to approach the hurdles faced by plasmid DNA vaccination [reviewed in Finn, 2003]. First, the nature and origin of immune response can be more finely tuned to the neoplasm by the site and target of immunization. For example, skin or muscle injection has been shown to confer systemic immunity in a variety of murine models involving cancer and infectious disease. Intramuscular injection results in plasmid DNA uptake by myocytes, which present antigen through major histocompatibility class (MHC)-I pathways [reviewed in Kutzler and Weiner, 2004]. Though these cells do not express costimulatory molecules to function as efficient antigen presenting cells (APCs), the plasmid gene is translated within the cell and the protein product excreted to initiate an antibody-based humoral-specific type of immune response. Professional APCs at the site of plasmid injection also serve to prime a cellular immune response through MHC-I pathways either by becoming transfected with DNA or through mechanisms involving crosspriming of antigen released by myocytes [reviewed in Donnelly et al., 2005]. Since a large degree of pathogens are transmitted across the mucosal epithelium, mucosal immunity might also be achieved through vaccination routes involving mucosal-associated lymphoid tissues (MALT) [reviewed in Hobson et al., 2003]. Advantages to MALT routes involving intranasal and oral vaccinations include priming at the site of infection and preventing the spread of disease to distal sites in the body.

A second strategy to improve the relative poor immunogenicity of DNA vaccines includes enhancing the level of immune response to a target antigen through, for example, the use of adjuvants (e.g., alum, cytokines, lipopolysaccharide) alongside DNA immunization. Adjuvants serve to activate innate immune cell

subsets such as natural killer (NK) cells in order to better promote adaptive immunity through T-cell interaction. Interestingly, bacterial plasmids contain unmethylated CpG motifs that help skew the immune response to a CD4+ T helper (Th)-1 type response by inducing the secretion of cytokines such as interleukin (IL)-6, IL-12, and interferon gamma (IFN-γ). The adaptive immune response might also be stimulated effectively through plasmid DNA fusion genes that encode for immunoenhancing elements such as cytokines [reviewed in Stevenson et al., 2004]. Additionally, multiple vaccination regimens (prime/boost modalities) hold potential for sustaining a high level response and eliciting several components of the immune system by first priming with DNA and subsequently boosting with an alternative vaccine strategy (e.g., protein antigen, viral vector encoding the antigen of interest). In this fashion, both a Th1 and Th2 response might result that could aid in tumor destruction through cell-to-cell responses and antibody-specific mechanisms such as antibody-dependent cell-mediated cytotoxicity. In the context of immune evasion, tumor cells can release molecules and factors that exhibit immunosuppressive effects and as a result of the tumor microenvironment could also be targeted in combination with immunization to achieve a heightened immune response level. For instance, increased occurrences of regulatory CD4+ T cells have been observed in patients with breast [Liyange et al., 2002; Wolf et al., 2003] and lung [Woo et al., 2002; Wolf et al., 2003] cancers. Abrogating the function of these cells in some fashion could promote systemic tumor immunity, and, indeed, mouse models have demonstrated tumor rejection following depletion of CD4+ CD25+ T cells [Casares et al., 2003; Yu et al., 2005]. In the area of immunoediting, vaccinating individuals against a wide array of tumor cell surface molecules could also help prevent the selection and outgrowth of tumors that could result from the use of plasmid encoding one target antigen.

A final approach to confronting the difficulties associated with DNA immunization includes initiating long-term memory to targeted antigens with DNA vectors in order to maintain systemic immunity. In this sense, a robust immune response is generated through active immunization with plasmid DNA, and after a targeted antigen is eliminated, a pool of memory T-cells is established to allow for rapid expansion of antigen-specific effector T-cells to prevent recurrence of a specific-cell population that expresses the target antigen [reviewed in Seder and Ahmed, 2003]. However, the details to achieve long-lived memory from a vaccine in a prophylactic or therapeutic scenario involving chronic antigen presentation are unclear to date and require further investigation.

CLINICAL APPLICATIONS OF DNA VACCINES

A number of murine systems have shown prophylactic success with reducing the spread of breast cancer through DNA vaccines. For instance, Luo et al. (2003) immunized mice with plasmid DNA encoding the transcription factor, Fos-related antigen 1, and *IL-18* gene transfected into *S. typhimurium*. Subsequent to oral immunization, mice were subcutaneously or intravenously (i.v.) challenged with a metastatic breast-carcinoma cell line. DNA immunization resulted in the breaking of tolerance to the encoded transcription factor protein, and through a MHC-I pathway involving CD4+ T cells, CD8+ T cells, NK cells, and dendritic cells, tumor angiogenesis, tumor cell growth, and metastasis was suppressed for the majority of immunized mice. Similarly, additional murine studies have shown immunity in breast cancer models incorporating tumor-associated antigens such as MAGE [Sypniewska et al., 2005] and Her-2/neu [Chang et al., 2004].

Studies in rodent models have been extended into clinical application, and, currently, one phase I clinical trial targeting breast cancer has been established in determining the safety and efficacy of DNA plasmid encoding the intracellular domain of Her-2/neu. The details of this study involved patients, in remission of disease after standard therapies, with stage II, III, and IV metastasis that were vaccinated with DNA and the cytokine adjuvant, granulocyte monocyte-colony-stimulating factor [Disis et al., 2004]. The majority of patients elicited both a specific humoral and cell-mediated response to Her-2/neu, and a specific immune response as a result of the vaccine was observed in some patients as long as 1 year after immunization. This suggested that in this scenario, DNA vaccination was successful in the elicitation of immunologic memory. However, the success of such DNA regimes must be considered within the context of preventing recurrence of disease over an extended period of time. To date, the FDA

approved monoclonal antibody, Tastuzumab (Herceptin), specific to metastatic breast cancers overexpressing Her-2/neu is an attractive alternative form of immunotherapy particularly in a combinatorial approach to chemotherapy to help reduce tumorigenic burden [reviewed in Kennedy and Shearer, 2003].

Prostate cancer remains the most common cancer among men in the United States with the median age of diagnosis at 69 [Ries et al., 2005]. For 2005, it is estimated that over 230,000 men will be diagnosed while more than 30,000 will succumb to the disease. As observed with targets to breast cancer, animal models have established immunity to prostate cancer when encoding plasmid DNA to a tumor-associated antigen [Qin et al., 2005; Roos et al., 2005]. Prostate-specific antigen (PSA) is a serine protease secreted at increased levels in prostatic neoplasms relative to normal tissues and is ideal from the standpoint of screening for the detection and recurrence of prostate cancer as well as representing a potential immunologic target for treatment. In the phase I clinical trial conducted by Pavlenko et al. [2004] patients with hormone-refractory prostate cancer were immunized against PSA alongside adjuvant cytokines. The results of the study indicated the efficacy of the DNA vaccine to induce both a CD8+ and CD4+ T cell response in the majority of patients that led to the reduction of observable PSA levels in the serum. Thus, the potential success of such a vaccine to prevent metastasis and occurrence of prostate cancer would implicate its use over the standard treatments of chemotherapy, radiation, surgery, and hormone therapy.

Lung cancer is the leading source of oncogenic death for both men and women yearly [Ries et al., 2005] with its most common cause being the result of exposure to tobacco and other harmful substances such as asbestos. Our laboratory has been actively involved in determining mechanisms of tumor immunity through DNA vaccination within a model of experimental pulmonary metastasis. We utilize a tumorigenic cell-line transformed by Simian virus 40 (SV40) that expresses a viral encoded tumor-specific antigen, large tumor antigen (Tag). SV40 Tag is an early expressed viral protein that aids in viral replication and cell transformation [reviewed in Butel and Lednicky, 1999]. Following the discovery that SV40 was a contaminant of poliovirus vaccines distributed in the United States between 1955 and 1963, SV40 Tag protein expression has been amplified through PCR in human malignant pleural mesotheliomas (MPM) [Carbone et al., 1994; Shivapurkar et al., 2000; Toyooka et al., 2001] along with a variety of other tumors [reviewed in Gazdar et al., 2002]. MPM arises in the pleura of the chest cavity and lungs and has been primarily associated with exposure to certain forms of asbestos. Upon diagnosis, most patients do not survive greater than 1 year after standard treatments of chemotherapy, radiation, and surgery. Yet, SV40's presence within MPM and role in pathogenesis remains unclear and controversial to date [Cristaudo et al., 2005; Manfredi et al., 2005].

In early studies from our laboratory, we have immunized mice with a DNA construct encoding the Tag gene under the control of the SV40 promoter, designated pSV3-neo. Within a solid murine tumor model whereby tumor cells were challenged intraperitoneally, DNA immunization resulted in protective tumor immunity that was associated with CD8+ T cell activation [Bright et al., 1996]. This DNA vaccination failed to generate an observable SV40-specific Tag-antibody response. Within a more stringent murine tumor model of experimental pulmonary metastasis [Watts et al., 1997], observable tumor cells were i.v. injected after pSV3-neo immunization [Watts et al., 1999]. Tumor burden within this model was determined through quantitation of tumor cell foci and survival, and only partial tumor immunity was observed and this was associated with a weak SV40 Tag-specific CD8+ T cell response.

To improve upon the level of tumor protection within our plasmid vaccination scheme, a second DNA vector was constructed that contained the SV40 Tag gene under the control of the stronger cytomegalovirus (CMV) promoter, designated pCMV-Tag [Lowe et al., 2005]. In a comparison study between the two plasmids, pCMV-Tag immunized mice elicited a SV40 Tag-specific antibody response along with a type-1 cytokine secretion profile that resulted in complete protection in both solid and experimental pulmonary metastasis tumor models. In vitro analysis of Tag mRNA and protein expression was also greater in cell lines transfected with pCMV-Tag. The results of this study indicated that the stronger CMV promoter induced greater gene expression of SV40 Tag, in relation to pSV3-neo, eliciting both a

cell-mediated and humoral-specific response to SV40 Tag in vivo. Interestingly, the importance of SV40 Tag antibody within this model of DNA vaccination and tumor challenge closely paralleled the requirement of CD4+ T cells and antibody in recombinant protein immunization of SV40 Tag [Kennedy et al., 2003]. Our laboratory is currently investigating the active immune cell subsets leading to tumor immunity in mice immunized with pCMV-Tag from both an induction and effector phase scenario to SV40 Tag expressing tumor cell challenge. Such an approach for determining the required cell components of the immune response allows for a deeper understanding of the events leading to systemic tumor immunity, and this knowledge could potentially be used to construct an immune-cell-specific DNA plasmid to vaccinate individuals predisposed to developing lung cancers expressing SV40 Tag such as MPM.

DNA VACCINES AS TOOLS AGAINST INFECTIOUS DISEASE

Much like the area of cancer, DNA vaccines remain a biological source for vaccination against infectious organisms to prevent the occurrence and progression of disease. Many similar challenges still face this level of work including the nature of immune response to vaccination. However, the strategies presented earlier, including prime/boost regimes and adjuvant administration, remain strategies to counteract such difficulties associated with DNA-based vaccines. Thus, plasmid DNA holds considerable promise as a tool for maintaining health in individuals likely to be at risk or exposed to pathogenic microorganisms, and work is ongoing in such areas involving Hepatitis [reviewed in Duenas-Carrera, 2004] and HIV-1 [reviewed in Giri et al., 2004] along with bacterial infections leading to Tuberculosis [reviewed in Haile and Kallenius, 2005] and meningitis [reviewed in Jodar et al., 2002]. It is beyond the scope of this present report to provide a detailed review as it relates to DNA vaccines against infectious diseases. Over a decade of investigations have examined DNA vaccination strategies that target human and veterinary infectious diseases, including viral, bacterial, and parasitic organisms. We will focus our discussion on studies that target some emerging viral infectious diseases that have the potential for worldwide spread.

The positive-strand RNA viruses of the genus *Flavivirus* are representative of a group of emerging infectious diseases that are of immediate concern to worldwide human health due to vector spread and infection as a result of human travel. Commercial vaccines for the mosquito-borne viruses causing Japanese encephalitis (JE) and yellow fever [reviewed in Marfin et al., 2005] exist. However, no vaccination method is available to help prevent the spread of disease associated with dengue (DEN) and West Nile (WN) viruses.

In light of the extremely successful yellow fever vaccine, there do remain a number of limitations with current JE vaccines. For example, short-term protection and severe adverse reactions have been observed with the readily available formalin-inactivated JE vaccine (JE-VAX) [reviewed in Mackenzie et al., 2004]. To counteract such issues, alternate candidate vaccines are undergoing development for international use in humans. Though JE DNA vaccines are in their infancy compared to the progress of DNA vaccine preparations against other infectious diseases, promising results have thus far been obtained. Plasmid DNA employing JE viral proteins in murine models have been shown to confer protection to JE due to neutralizing antibody [Konishi et al., 1998; Chang et al., 2000; Pan et al., 2001] and cytotoxic T-lymphocytes (CTLs) [Konishi et al., 1998]. Interestingly, data reported from both murine [Chang et al., 2000] and non-human primate systems [Tanabayashi et al., 2003] indicated that the level of protection from JE challenge using a DNA construct was as effective as those animals immunized with the commercially available JE-VAX preparation. Additional work within this area is ongoing in order to enhance the level of immune response and protection to JE challenge through varying immunization regimes involving plasmid DNA [Chen et al., 2005; Imoto and Konishi, 2005].

Protection from DEN virus infection through vaccination has proven particularly troublesome as no cross-protection exists between the viruses' four related but serologically distinct subtypes (DEN-1, DEN-2, DEN-3, DEN-4). One group of investigators has shown partial protection in mice immunized with plasmid DNA encoding a domain of the envelope protein from all four DEN serotypes upon viral challenged with DEN-2 [Mota et al., 2005]. Non-human primate studies have also reported a high

degree of protection from DEN-1 infection by immunizing with plasmid DNA against premembrane and envelope viral proteins in combination with immunostimulatory molecules [Raviprakash et al., 2003]. However, a successful DEN immunization regime must include and confer protection from all subtypes since it is possible to be infected concurrently with each distinctive DEN virus and the associated pathogenesis can result in hemorrhagic disease.

Within the past several years, WN virus has become a health concern primarily to immunocompromised and elderly individuals in the northern hemisphere. Yet data from vaccination studies provide insight into the potential success DNA immunizations may have in preventing WN infection in humans. Davis et al. [2001] have shown that mice and horses immunized with a plasmid vector encoding for both WN premembrane and envelope proteins developed WN-specific neutralizing antibodies and were completely protected from viral challenge. In an additional study, the source of protection in mice resulting from a plasmid DNA preparation, encoding the WN capsid gene, was determined to involve both an antibody and T-helper-1 response, including the secretion of IFN-γ and IL-2 cytokines [Yang et al., 2001]. Relative to human application, a phase I clinical trial sponsored by the National Institute of Allergy and Infectious Diseases is currently underway in healthy volunteers to test the safety and immunogenicity of plasmid DNA encoding for WN precursor transmembrane and envelope proteins.

CONCLUSIONS

The inherent nature of plasmid DNA from both a structural and immune response aspect, affords it great potential as a vaccine and preventative treatment in areas involving cancer and infectious disease. Though studies in the past have adopted this vaccination strategy as clinically ineffective and problematic due to its low immunogenicty, we now are aware of techniques to boost the level and skew the nature of the immune response. However, additional studies must be performed so that vaccination regimes involving plasmid DNA can be specifically tailored in order to mount an effective immune response against transmissible and non-transmissible diseases. With these thoughts in mind, the area of DNA vaccines is continuing to advance at a progressive rate with the great possibility to improve the quality of life of individuals at high risk to or stricken with disease in which successful vaccines or therapies do not presently exist.

ACKNOWLEDGMENTS

This work was supported in part by grant RR-12317 from the National Institutes of Health.

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