6. DNA VACCINATION IN IMMUNOTHERAPY OF CANCER

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INTRODUCTION

Nucleic acid immunization has garnered much attention as a promising approach for cancer therapeutic development. This innovative vaccine strategy uses non live, non replicating, non spreading DNA formulations which utilize the host's cellular machinery for expression of proteins (antigens). Such novel delivered and expressed antigens become recognized by the host immune response and induce specific T and B cell responses against the gene encoded proteins. The foundational basis for DNA vaccines originated from the observation that delivery of gene sequences in vivo could lead to their expression [reviewed in (1)]. In the 1950s and 1960s experiments aimed at understanding the fundamental nature of the basis for cancer delivered as either nucleic acid or proteins to animals and followed tumor development. Tumor development segregated with nucleic acids and tumor bearing animals could seroconvert to tumor antigens, establishing the ability of nucleic acid transfer to drive protein expression and activate the immune response. In the 1980s the understanding that the immune response was a nemesis for gene therapy antigen delivery started to impact vector studies. Wolff et al. reported that activity of reporter genes could be detected for up to two months without a delivery system (2). Many investigators were focusing on if such proteins implemented for such gene therapy experiments could be employed to express antigens to stimulate the immune system. A study

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published by Tang et al. utilizing DNA coated gold microprojectiles to transfect cells *in vivo* reported the generation of an antibody response against the DNA encoded proteins (3). The study utilized a delivery system called a gene gun as this group had doubts that the efficiency of direct injection was likely substantial enough to deliver enough antigen to produce a significant immune response. However, papers appeared from Ulmer and Wang almost coincidently (4, 5) describing different formulations for IM delivery inducing responses against true human pathogens, influenza and HIV, followed rapidly by a paper from Fryan on influenza. These papers demonstrated that not just antibodies could be induced by this technology, but that cellular response as well as cytokine profiles could also be altered. In addition, protection from animal challenge could also be achieved.

Among the most intriguing and yet promising aspect of DNA vaccines is the feasibility of manipulation towards benevolent immune responses, juxtaposed with the remarkable ability to activate both arms of the immune system (4, 5). These unique attributes are a result of direct transfection of encoding plasmids into cells *in vivo*, whereby direct presentation on MHC Class I can materialize. As will be discussed later, this very principle has been exploited to specifically amplify the cellular arm of the immune response. The other beneficial aspect is its ability to engineer responses robust enough to often break tolerance against nonimmunogenic tumor antigens. This is particularly important when dealing with antigens exhibiting minimal immunogenic properties. In view of these findings, we review the potential utility of applying DNA vaccines as both a therapeutic and prophylactic approach against cancer and other forms of neoplastic growth. The requirement of potent T-cell activation rather than antibody activation as an essential criterion for tumor rejection validates such a use for efficacious treatment for tumor immunotherapy (6).

ESSENTIAL ROLE OF DENDRITIC CELLS IN TUMOR IMMUNOGENICITY: OPTIMIZING TO TARGET DC'S

In the body's immune system, cells need to process and present antigenic peptides to lymphocytes in order to stimulate antigen specific immune response. Thus, antigen must be processed and presented to T lymphocytes by antigen presenting cells (APCs) (7). Antigen presentation and recognition is a complex biological process that involves many interactions between antigen presenting cells and T cells. There are four primary components that are critical in the professional APCs' ability to present the antigen to T cells and activate them for appropriate immune responses. These components are MHC-antigen complexes, costimulatory molecules (primarily CD80 and CD86), intracellular adhesion molecules, and soluble cytokines. Naive T cells circulate through the body across lymph nodes and secondary lymphoid organs such as the spleen. Their migration is mediated among other factors by intercellular adhesion molecules and cytokines. As the T cells travel, they bind to and dissociate from various antigen presenting cells (APCs). Their movement is guided by chemokines but binding to cells is mediated through adhesion molecules. When a naive T cell binds to an APC expressing relevant MHC:peptide complex, the T cell

up regulates costimulatory molecules such as CD40L which further activates the APC increasing expression of the T cell costimulatory molecules CD86/CD80. These costimulatory signals bind to CD28 on the T cells inducing increased levels of high affinity IL-2 receptor. Only when this T cell receives a strong enough costimulatory signal through CD80/CD86-CD28 interaction does the T cell make soluble IL-2, which then binds to the receptors and drives the now-armed effector T cell to activate and proliferate.

Much speculation on the immunogenicity of tumor antigens has concentrated on the efficiency of antigen presentation from APCs to T-cells. Specifically, the roles of Dendritic cells (DCs) and the essential antigen specific clonal expansion have been thoroughly investigated. When compared with other "professional" antigen presenting cells (APCs), dendritic cells are preferentially advantageous due to their exclusive ability to activate naïve T-cells within the secondary lymphoid organs. Structurally, dendritic cells express elevated quantities of co-stimulatory molecules including CD80 and CD86, and large amounts of peptide-MHC complexes allowing potent T lymphocyte activation and differentiation (Reviewed in 8).

The role of DC's in regulating tumor antigenicity have been extensively documented. In fact, tumors have developed immune evasive attributes to prevent DC maturation and prevent the eventual antigen specific T-cell generation. For instance, some tumors secrete substantial amounts of Vascular Endothelial Growth Factors (VEGF) which promote not only angiogenesis but also retards the maturation of antigen captured DC's (9) and this effect appears to operate through a direct suppression of NF-κB (10). Furthermore, continuous infusion of VEGF into mice leads to a dramatic inhibition of dendritic cell development, associated with an increase in the production of B cells and immature Gr-1(+) myeloid cells (11). In addition to VEGF, some tumor cells secrete IL-10, which directly prevents the maturation of dendritic cells (12, 13). This effect dramatically diminishes the antigen specific activation of both CD4 and CD8 T cells through the attenuation of DC's, as *ex vivo* generation of bone marrow-derived DC eradicates this effect (14). These results suggest a paramount theme; tumors suppress the maturation of DC's to impede the functional stimulation of T cells. Unfortunately for the development of anti tumor immunity, this effect is essential for proficient activation of tumor specific T cells.

Essential Role of DCs in DNA-based Immunizations

In addition to anti-tumor immunity, the development of DNA vaccine-based immune responses also requires the activation and and perhaps in part direct transfection of dendritic cells. Condon et al. demonstrated that gene gun delivery of reporter gene plasmids to the skin resulted in expression of the reporter genes in cells exhibiting dendritic cell-like morphology localized within the local draining lymph nodes (15). Others have proposed that secretion of expressed antigens from somatic cells or their destruction (i.e. muscle cells and keratinocytes) may provide the path for dendritic cells to take up antigen and present through the exogenous MHC class II pathway (16). In fact purification of APCs from immunized mice will stimulate naïve T cells in an antigen specific manner, suggesting that uptake

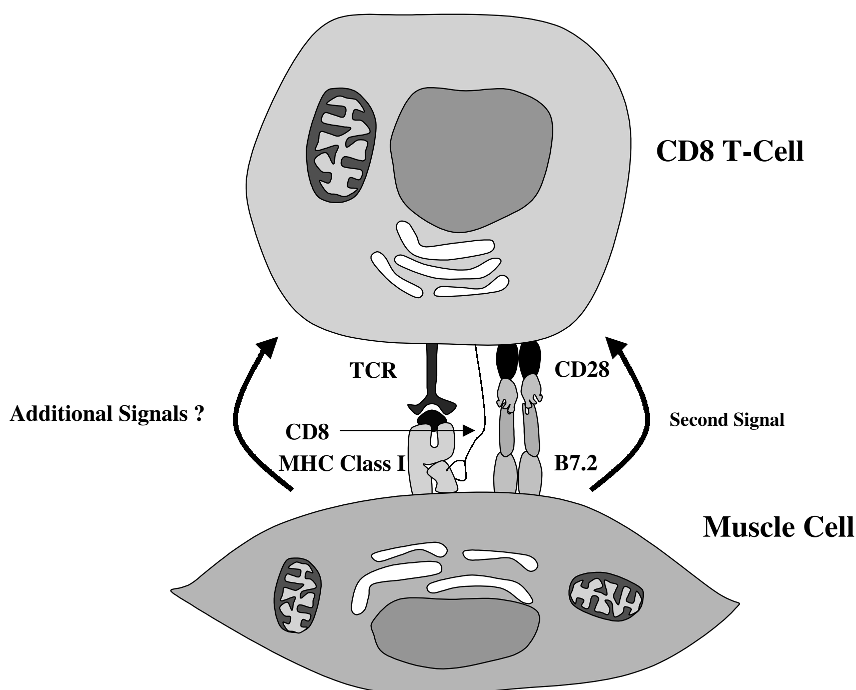

Figure 1. Muscle Cells as Antigen Presenting Cells (APCs). A schematic diagram of the *in vivo* expression of B7 surface molecules on muscle cells. The direct transfection of B7 activates the secondary signal by signaling through CD28 to activate MHC mediated presentation and activation of T-cells. This coimunization model induced potent antigen specific CTL activation with muscle cells functioning as APCs.

of antigen by APCs is important for DNA immunization based immune activation. Additionally, IM injection of plasmids expressing EGFP results in co-localization of EGFP expressing cells and APC markers CD80 and CD86 in the draining LN's (17). These results suggest that 1) APC activation and migration to the regional lymph node is essential for immune activation following plasmid immunization, and 2) APCs can take up antigens through direct transfection and/or exogenous phagocytosis. Therefore, vaccine strategies targeting the enhanced uptake of antigens by DCs or increased chemotaxic migration of DCs to the site of antigen expression may provide increased vaccine potency and increased therapeutic potential.

Enhancing DC-directed Antigen Uptake

In addition to powerful signaling, dendritic cells often function as scavenger cells by engulfing and processing apoptotic bodies. Specifically, immature DCs uptake dead cells or apoptotic bodies via surface receptors $\alpha V\beta 5$ integrin and CD36 (18–20). This uptake promotes a process termed cross-priming whereby exogenous antigens are processed and presented through the endogenous MHC class I pathway (Figure 1).

Specifically, the engulfment of these bodies with either tumor or viral antigens by dendritic cells provokes the activation of MHC class I restricted CD8+ CTLs (20, 21). Both dendritic cells and macrophages have been shown to present apoptotic engulfed antigens but the latter is much less potent at activating naïve T-cells, which is a vital step in the generation of adaptive immunity (21, 22). Furthermore, several studies also demonstrate that there is a quantitative dependency on apoptotic bodies by dendritic cells in inducing the secretion of pro-inflammatory cytokines TNF-α and IL-1β both *in vitro* and *in vivo* (20, 23). Accordingly, an optimum strategy to develop potent vaccines would include the activation of dendritic cells and the packaging of immunogens in apoptotic bodies facilitating cross priming and broader cellular immunity. In a recent study, a novel strategy was used whereby immunogen constructs were coimmunized with an apoptosis inducing receptor Fas. This coimmmunization resulted in significant augmentation of antigen specific immune responses as measured by enhanced CTLs and Th1 cytokines including INF-γ and IL-12 (24) (Figure 2). There is other evidence to suggest that apoptosis signals that aliquot ample time for immunogen expression will provide adjuvant properties. A more recent study implemented mutant caspases to decrease apoptotic efficiency to increase the time of immunogen expression prior to the apoptotic event, while still delivering apoptosis-mediated antigens to dendritic cells (25). This specific adjuvant raised both CD4 and CD8 responses, indicating that antigen uptake by DCs presented peptides into both the endogenous and exogenous pathways.

While apoptosis mediated delivery has provided an insight to the possibility of cross priming, others have utilized directly secreted antigens to target dendritic cells. This raises the prospect that exogenous antigens may function in generating MHC Class I-restricted responses by directly entering the cytosol in a DNA vaccine model through the endogenous cross-priming process (26–28). It has been previously suggested that antigens can be expressed by transplanted cells, which maintain the ability to induce CTLs through the direct transfer of antigens to host's antigen presenting cells (29, 30). Various tumor studies directed at ascertaining the precise functions of somatic MHC class I molecules have determined that bone marrow-derived antigen-presenting cells play the dominant role of presenting these somatic-based antigens (31). Additionally, other exogenous antigens such as bacteria are internalized and processed for presentation by MHC class I molecules (32, 33). These reports unequivocally imply extracellular uptake of antigens is a prominent pathway implemented by the immune system to generate CTLs. In this regard, dendritic cells express surface cell receptors for Fc regions of antibodies called FcγR's, which enhances the uptake of antigen-antibody complexes and leads to presentation on MHC class II molecules (34). These receptors also assist in the activation and maturation of dendritic cells and regulate efficient presentation of exogenous antigens (35). Hence introduction of exogenous antigens through FcγR-mediated internalization into the cytoplasm may help effectively prime antigens to activate MHC Class I-restricted CTLs (36). Exogenous antigen endocytosis could then employ the endogenous TAP dependent antigen processing pathway and in theory present peptides on MHC Class I molecules (37). This strategy was directly tested in a DNA vaccine model by fusing

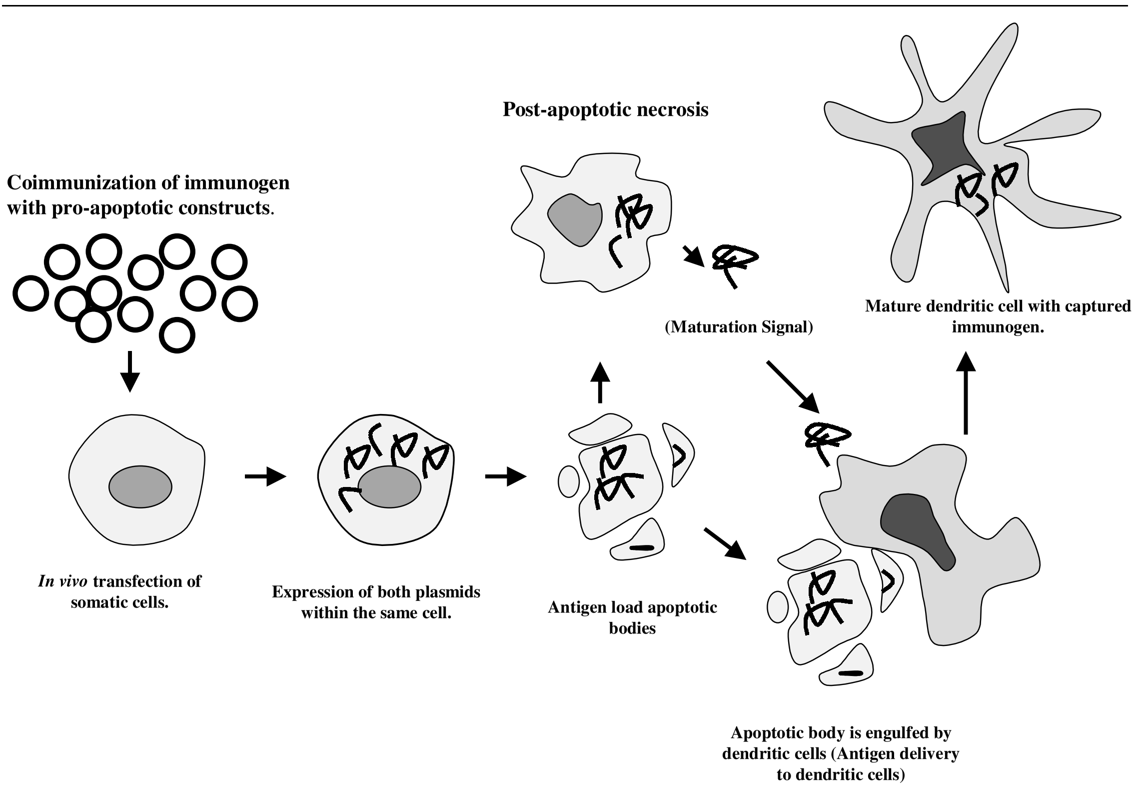

Figure 2. Apoptosis-mediated Delivery of DNA Vaccines. The following model describes a possible mechanism by which antigen loaded apoptotic bodies deliver and induce maturation of dendritic cells. As shown, potent and rapid induction of cell death generates apoptotic bodies loaded with antigens to specifically deliver to antigen presenting cells. Late apoptosis or necrotic bodies may provide essential signals need to induce the maturation.

the hepatitis B virus (HBV) e antigen and the Fc portion of an IgG1 antibody (38). The antigen-Fc immunogen was secreted by somatic cells and taken up effectively by dendritic cells resulting in stimulation of both CD4+ and CD8+ T cells *in vivo*. The adjuvant effectively augmented the secretion of proinflammatory cytokines including INF-γ and IL-2 as well as enhancing CTL and lymphocyte proliferative responses (38).

Another promising DC targeting immune modulator is the family of chaperones called the Heat Shock Proteins (HSPs). The initial experiments that elucidated the immunocapability of HSPs were from purification experiments from antigenically distinct sarcoma cells (39, 40). In fact, it was later ascertained that this 96 kDa glycoprotein was not itself immunogenic, but became immunogenic in circumstances when it was conjugated with peptides. Overall comparison of immunogenicity with other HSP family members including Hsp70 and Hsp90 suggest that immunogenicity is associated with two vital factors, the associated ATPase activity and the association of HSP with peptides (41). The ATPase activity likely determines the ability of the chaperone complex to transfer peptide to acceptor molecules and this association with peptides is the rationale for the autologous nature of these complexes (42). These chaperones also possess intrinsic inflammatory qualities, including among many properties the maturation and activation of DCs (43), direct cross-priming abilities (44–46), and release of NO from APCs (47). The endocytosis and eventual presentation of antigens is thought to be a consequence of the universal targeting of all HSPs by its receptor CD91, a natural ligand for alpha 2-macroglobulin (48, 49). The post uptake processing implements the endogenous pathway, which partially explains the cross-priming effect of these proteins. The significance of HSP in tumor-specific and non-vaccine related circumstances has been examined. Specifically, immunotherapy of cancers with HSPs purified from tumors or reconstituted *in vitro* from tumor cell cultures when administered as vaccines also regressed the growth of tumors (50–52).

The copious immunogenic attributes of HSP suggest these complexes may function as useful adjuvants for DNA vaccines. Specifically, the HSP70 of Mycobacterium tuberculosis was fused to the Human HPV-16 E7 antigen generating a chimeric DNA vaccine (53). The E7-HSP70 DNA vaccine induced significantly enhanced levels of Th1 mediated responses including a ratio of 435:14 (E7-HSP70 to E7) of E7 specific INF-γ spot-forming CD8+ T cells via ELISPOT assays. Additionally, data from this group suggested that the eradication of pre-existing tumors and the resulting immune response was via CD4+ independent mechanisms, implying that cross-priming was crucial for this effect. Where the T cell help for this cross priming event was supplied is unclear.

Another member of the HSP family that has shown to augment the potency of DNA vaccines is calreticulin (54). The idea of calreticulin as an immune modulator was based on previous findings that calreticulin in conjugation with tumor peptides stimulates potent peptide specific CD8+ T cell responses (55). Like the other HSP members, calreticulin also implements the CD91-dependent pathway for APC uptake, making cross-priming another presumable immunogenic outcome from use

of this molecule (49). Additionally, calreticulin and its fragment vasostatin also operate as inhibitors of angiogensis (56, 57). Accordingly, when calreticulin was fused to HPV-16 E7 antigen as a DNA vaccine, a potent, anti-tumor effect was provoked; the resulting response was attributed to both the enhanced immunogenicity against E7 and the generation of anti-angiogenesis.

Amplifying DCs at the Site of Expression

In addition to the enhanced uptake of antigens, an amplification of DC quantity is likely advantageous for enhanced antigen delivery to and presentation within the regional lymph nodes. Compensating for DC paucity can be ameliorated through DNA vaccine-mediated engineering of immune responses. Among various strategies, Flt3 (Fms-like tyrosine kinase 3) ligand (FL) has been utilized to stimulate the activation and amplification of dendritic cells (58). Functionally, FL treatments in mice significantly increase dendritic cell population in many areas including the bone marrow, gastro-intestinal lymphoid tissue (GALT), liver, lymph nodes, lung, peripheral blood, peritoneal cavity, spleen, and thymus (59). FL has revealed tumor suppressive attributes in mice partly by the generation of large number of dendritic cells (60). Accordingly, recent work indicates enhanced frequency of CD8+ T cells when FL was fused to the human papillomavirus-16 E7 antigen (58). The response was CD4+ independent and maximum effect was observed when the antigen and FL were fused together. Most importantly, 100% of mice vaccinated with FL-E7 were protected when challenged with TC-1, a tumor cell line derived from C57BL/6 mice cotransformed with HPV-16 E6 and E7 and c-Ha-ras oncogenes (58).

Other methods have concentrated on the direct chemotatic migration of DCs to the site of injection. One report specifically coimmunized M-CSF and resulted in enhanced levels of CD8+ T cell dependent responses. This effect was in direct correlation with elevated DC migration to the site of injection with an increase in the Beta-Chemokine MIP-1β (61). Others have reported that coimmunization with GM-CSF augments the migration of immature DCs to the injection site. The maximal migration occurred between days 3–5 post injection and was not positive for CD80 or CD40 (62).

CONVERTING MUSCLE CELLS INTO APCS

The generation of immune responses via DNA vaccines requires the delivery and/or presentation of immunogens to professional antigen presenting cells (APCs). It has been proposed that a significant target for *in vivo* transfection of plasmids are also the muscle cells themselves and their transfer and/or presentation of immunogens may be a vital tool in the development of immune responses (Reviewed in 63). One of the consequences suggested as an issue for muscle delivery of plasmid vectors is that less effective antigen presentation will occur as the muscle cells fail to express the costimulatory molecules (i.e. CD80, CD86, B7RP1) necessary to send a second signal. One approach to this limitation may be to codeliver antigen expressing plasmids with costimulatory molecule expressing plasmids (64–66). In theory the coexpression of CD86 on muscles that express a high ratio of MHC class I and can

not a highly invasive approach which exploits the host's own immune system to generate intrinsic anti-tumor defenses. In addition, it can be easily combined with other approaches thus is attractive to patients and physicians. Additionally, side effects associated with conventional chemotherapy and radiotherapy are nonexistent with immunotherapies. To this end, several specific TAAs have become targets for DNA vaccine development (reviewed in 79, 80).

DNA Vaccines Against Melanoma

An imposing barrier for any immune therapy approach is the potential for immune tolerance to tumor antigens. It has been suggested that the unique presentation of tumor specific antigens in the context of DNA vaccines may facilitate breaching of this potential immunological barrier. Among the numerous TAAs that have been identified for melanoma, several have been studied in the context of DNA vaccines. Specifically, an early experiment conducted by Weber et al. targeted the gp75/ tyrosinase-related protein-1 as an antigen in a DNA vaccine model. This specific TAA is well tolerated as a vaccine antigen although it is difficult to develop strong cellular immunity against. However, mice primed with the human gp75 and boosted with murine gp75 appear to be able to break tolerance and developed immunity and tumor protection in a mouse challenge model. This immune response was dependent on both the induction of CD4+ T cells and NK cells (81). Another innovative strategy used minigenes as specific targeted immunogenes, by fusing distinct dominant class I epitopes from gp100 and TRP-2 into the vaccine candidate. In addition, the ubiquitin gene was fused on the 5′ end of the vaccine and was delivered by oral gavage using an attenuated strain of *Salmonella typhimurium* as carrier. The enhanced effect was concomitant with increased INF-γ production and specific lysis of tumor cells by activated CD8 T cells. The effect is thought to be a consequence of increased processing and targeting of the antigen into the MHC Class I presentation pathway (82). Several studies have tested plasmid vaccines against different models of melanoma. Some of these studies induced destruction of pigment cells as a possible correlate of destruction of melanoma *in vivo*. However, the early results appear to just be scratching the surface. It is likely that more potent DNA vaccines incorporating molecular adjuvants or in prime boost protocols will be more effective than these early approaches.

DNA Vaccines Against Colon Cancer

Human CEA is a 180-kDa glycoprotein expressed in elevated levels in 90% of gastrointestinal malignancies, including colon, rectal, stomach, and pancreatic tumors, 70% of lung cancers, and 50% of breast cancers (83, 84). CEA is also found in human fetal digestive organ tissue, hence the name carcinoembryonic antigen (85). It has been discovered that CEA is expressed in normal adult colon epithelium as well, albeit at far lower levels (86, 87). Sequencing of CEA shows that it is associated with the human immunoglobulin gene superfamily and that it may be involved in the metastasizing of tumor cells (85).

The immune response to nucleic acid vaccination using a CEA DNA construct was characterized in a murine model. The CEA insert was cloned into a vector containing the cytomegalovirus (CMV) early promoter/enhancer and injected intramuscularly. CEA specific humoral and cellular responses were detected in the immunized mice. These responses were comparable to the immune response generated by rV-CEA (86). The CEA DNA vaccine was also characterized in a canine model, where sera obtained from dogs injected intramuscularly with the construct demonstrated an increase in antibody levels (88). Cellular immune responses quantified using the lymphoblast transformation (LBT) assay also revealed proliferation of CEA-specific lymphocytes. Therefore a CEA nucleic acid vaccine was able to induce both arms of the immune responses (88). CEA DNA vaccines are currently being investigated in humans, but as yet there is little data presented for guidance.

DNA Vaccines Against Prostate Cancer

Prostate cancer is the most common form of cancer and the second most common cause of cancer related death in American men (89). The appearance of prostate cancer is much more common in men over the age of fifty (90). Three of the most widely used treatments are surgical excision of the prostate and seminal vesicles, external bean irradiation, and androgen deprivation. However, conventional therapies lose their efficacy once the tumor has metastasized, which is the case in more than half of initial diagnoses (91, 92).

PSA is a serine protease and a human glandular kallikrein gene product of 240 amino acids, which is secreted by both normal and transformed epithelial cells of the prostate gland (93, 94). Because cancer cells secrete much higher levels of the antigen, PSA level is a particularly reliable and effective diagnostic indicator of the presence of prostate cancer (95). PSA is also found in normal prostate epithelial tissue and its expression is highly specific (96).

The immune responses induced by a DNA vaccine encoding for human PSA has been investigated in a murine model (96). The vaccine construct was constructed by cloning a gene for PSA into expression vectors under control of a CMV promoter. Following the injection of the PSA DNA construct (pCPSA), various assays were performed to measure both the humoral and cellular immune responses of the mice. PSA-specific immune responses induced *in vivo* by immunization were characterized by enzyme-linked immunosorbent assay (ELISA), T helper proliferation cytotoxic T lymphocyte (CTL), and flow cytometry assays. Strong and persistent antibody responses were observed against PSA for at least 180 days following immunization. In addition, a significant T helper cell proliferation was observed against PSA protein. Immunization with pCPSA also induced MHC Class I CD8+ T cell-restricted cytotoxic T lymphocyte response against tumor cell targets expressing PSA. The induction of PSA-specific humoral and cellular immune responses following injection with pCPSA was also observed in rhesus macaques (97). These responses were achieved in either female or male animals. As the PSA construct was human in design, and human and rhesus construct are 98% identical these results support that the DNA vaccines could break tolerance in this model. This is a rare demonstration

of this ability in a non human primate. In addition to cellular immunity, strong antibody responses were also observed. Such antibody responses may also be valuable in a clinical setting. Recently, PSMA based DNA vaccines have entered the clinic for initial evaluation. The results of these studies are pending but will likely provide important information about targeting prostate disease using DNA technology.

DNA Vaccines Against Cervical Cancer

Human Papillomavirus (HPV) 16 associated proteins including E6 and E7 are some of the most common proteins in cervical cancers and are ubiquitious expressed within these cells (98,99). However, DNA based vaccine targeting these proteins seem to elicit minimal immune responses and may necessitate potent adjuvants to provide efficacious tumor protection. A DNA vaccine based HPV E6 vaccine in mice was able to provide anti-tumor activity when adjuvanted with IL-12 into the skin. This specific study implemented exclusively the amino terminal which of E6, which lacks the transforming property (100). One of the early E7 vaccines employed mutational variants within the zinc-binding motifs that led to rapid degradation. Ironically, this specific vaccine exhibited stronger E7-specific CTLs (101). In a similar fashion, several other studies have targeted the processing of HPV vaccines to specific compartments to enhance potency. An early study by T.C. Wu and colleagues fused the E7 antigen with the lysosomal-associated membrane protein (LAMP-1), which directs processing of E7 antigens into the MHC class II pathway for presentation (102). When compared to the E7 antigen alone, the LAMP-1 mediated targeting enhanced antigen specific CD4+ helper T cells, greater antigen specific E7 CTL activity, and antibody responses (103). On the contrary, similar manipulation has been implemented to direct proteins into the MHC class I presentation pathway. Specifically, the HSV-1 structural protein VP22, which exhibits an intercellular trafficking property (104), was directly fused to the E7 antigen to perhaps increase presentation productivity (105). Incredibly, this specific adjuvant stimulated a 50-fold increase in the overall quantity of E7-specific CD8+ T cells (105). Similarly, fusion of E7 to gamma-tubulin, a target for the centrosomal compartment which possesses proteasomes, led to a dramatic increase in the quantity of E7-specific CD8+ T cells. This effect was dependent on the proteasome, as mice deficient in TAP-1 failed to develop such an enhancement (106).

A more recent report within the clinics also suggests that immunization through DNA can also therapeutically attenuate the growth of neoplastic cells in humans. These studies specifically encapsulated DNA plasmids encoding HLA-A2-restricted epitopes of the HPV E7 antigen within biodegradable polymer microparticles. Early work suggests no adverse side effects, while enhancing immune responses when implementing this specific therapy (107). These results are very exciting as the doses of DNA used in these studies are very low. In addition to the demonstration of immune response induction these investigators noted a regression in precancerous phenotype in this cervical progression model. While spontaneous regressions are noted in this model, the rate of regression gives hope that this regression was a result of the vaccine. However, strong conformation of these results will await a

clinical study that includes a placebo control to firm up these important observations. However, the results remain highly exciting and may mark a turning point in the application of this technology to cancer therapy of Papillomavirus infection.

DNA Vaccines Against Breast Cancer

The *erbB-2/neu* proto-oncogene is a member of the EGFR family that dimerizes to activate *trans* phosphorylation to activate signal transduction and is also overexpressed in 15–40% of all human breast cancers (108–110). Accordingly, Chen et al. generated DNA constructs expressing the full length *neu*, the extracellular domain, and the extracellular-transmembrane domains. The latter two mutants were created to avoid potential transformation, and all three were immunized and challenged with Tg1-1 cell line, which was garnered from a FVB/N *neu*–transgenic mouse. The authors report protection when challenged with this specific cell line and this effect was augmented with IL-2 as an adjuvant, and was antibody independent (111). Others have implemented innovative strategies by mutating domains responsible for kinase activity and adding leader sequences to redirect towards antigen processing and have generated similar results (112). Importantly, the prophylactic attributes of this vaccine was demonstrated when it was shown to prevent spontaneous formation of tumors in FVB/N *neu*-transgenic mice when administered in conjunction with IL-12 (113). One concern for clinical evaluation of this approach is that neu is expressed in many other tissues besides breast cancer, including lining of the brain and in heart tissue, at low levels. The consequences of this expression for DNA immune therapy is at this time unknown but must be considered in clinical trial design.

Conclusion

The recent progress of immunotherapy for treatments against cancer can largely be attributed to a greater overall understanding of the immune system. Identification of processing pathways and targeting receptors has allowed the development of novel adjuvants in augmenting the overall potency of these vaccines. In addition, the growing lists of TAAs provide copious targets to develop immunity against tumor formation. Furthermore, therapies can also target factors that are essential for the survival and propagation of tumors. For instance, a recent study targeted the receptor of the angiogenesis factor VEGF. The authors immunized mice against vascular-endothelial growth factor receptor 2 (FLK-1) through an oral vaccine and targeted proliferating endothelial cells in the tumor vasculature. Protection was observed from numerous cell types including melanoma, colon carcinoma, and lung carcinoma (114). Accordingly, this combination of basic immunology and TAA isolation is providing an auspicious path for immunotherapies against cancer. All together, these promising results also emphasize the potential of DNA Vaccines as therapies against cancer. The particular advantages of DNA in manufacturing, lack of replication based pathogenesis, specificity for the tumor target, lack of vector immunity allowing for routine reimmunization are all properties of ideal immunization strategies for cancer

immune therapies. The challenges as we go forward will be to take these collection of positive attributes and add additional immune potency to the mix. At that time it is likey that DNA vaccines will take their place at the center of programs for tumor immunotherapy.

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