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Review

DNA Tumor Vaccines

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Abstract. A new generation of vaccines are being developed to induce immune responses that fight off infectious agents, or erradicate cancerous cells. These new vaccines are based on a plasmid vector, which in transfected mammalian cells cause constitutive high-level expression of the target antigen. Expression of the target antigen, in turn, can induce a full-range of immunologic responses, including cell-mediated killing, cell-mediated cytokine release and the production of antigen-specific antibodies. Through molecular techniques, these nucleic acid vaccines can be enhanced to increase target antigen expression and facilitate antigen presentation. Additionally, genetic adjuvants expressed simultaneously with the target antigens can induce the immune responses to disease--associated antigens. The ease with which these genetic vaccines can be generated and the potency of their ability to generate immune-mediated responses make them highly effective, which creates hope for developing effective treatment and prevention of various diseases, most notably cancer.

Key words: DNA vaccine; T cells; cancer; immunity; cytokines.

Introduction

Vaccines can be considered as the foremost achievement of biomedical research in the 20th century. Vaccines to some viruses, such as smallpox and poliovirus, have been so efficacious that world-wide vaccination programs have accomplished complete or near complete eradication of human infections. In comparison, tumor vaccines have yielded far less impressive results. This relates to the complexity of the molecular changes that transform a "normal" cell into a malignant one. Tumor vaccine development is further complicated by the indoctrination of the immune system to avoid horror autotoxicus by destruction of self.

With advances in the human genome project, tumor--associated antigens are now being identified at a rapid pace (Table 1). Many of these antigens are either mutated or overexpressed self proteins that, in their wild--type form, play a role in cell cycle control. Single point mutations, such as those found in tumor-associated p53, that distinguish a normal protein from an oncoprotein, can serve as targets for T cell-mediated immune responses, provided the mutations are flanked by anchoring sequences for binding to major histocompatibility complex (MHC) determinants which are required to present the epitopic protein fragment to T cells[97]. Many mutations lack those sequences and are thus immunologically silent. Another commonly found molecular digression in tumor cells is over-expression or developmentally aberrant expression of otherwise unmodified self-proteins. The immune system is tolerized against self-proteins expressed at physiological levels. Induction of T cell tolerance requires a certain threshold of epitopes, which is formulated by a number of variables,

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Table 1. Genes expressing tumor-associated antigens

Antigen	Reference
MelanA/MART-1	41
pmel-l7/gp100	19
tyrosinase	45
gp75/TRP-1	99
TRP-2	98
MAGE-1	95
MAGE-3	29
GAGE-1, -2	94
BAGE	4
RAGE	79
NY-ESO-1[a]	11
CDK-4	104
β-catenin[b]	73
MUM-1	18
caspase-8[b]	40
KIAA0205[c]	32
SART-1	83
PRAME[d]	51
p15[d]	70
SSX1/SSX2[e]	20
Ep-CAM/EGP40[f]	66
MUC1[g]	57
GCAP	85
hsp70, hsp90, gp96/grp94[h]	35

MART-1 – melanoma antigen recognized by T cells 1, gp100 – melanoma differentiation antigen, tyrosinase – melanin synthesis enzyme, TRP-1, TRP-2 – tyrosinase-related protein, MAGE-1, MAGE-3, GAGE-1, GAGE-2, BAGE and RAGE – melanoma antigens (the suffix "AGE" in the name of the antigen coding genes indicates they are silent in normal adult tissues except testis), CDK-4 – cyclin-dependent kinase 4, MUM-1 – melanoma ubiquitous mutated, SART-1 – squamous cell antigen recognized by T cells 1, Ep-CAM – 17-1A recognized antigen, MUC1 – mucin, GCAP – germ-cell alkaline phosphatase, hsp70, hsp90, gp96/grp94 – tumor-derived stress proteins.

[a] A testicular cancer antigen

[b] Expressed as a mutated gene product.

[c] A human bladder cancer antigen.

[d] Melanoma – specific antigens.

[e] A translocation/fusion with the *SYT* gene (human chromosome 18).

[f] Up-regulated on several types of carcinoma.

[g] Up-regulated and/or under-glycosylated expression on certain adenocarcinomas.

[h] Tumor-derived stress proteins complexed with tumor-derived antigens.

including the level of protein production and turnover rate and the affinity of potential epitopes to MHC determinants. High-affinity epitopes are generally presented in sufficient quantities to the immune system to induce tolerance even if the self-protein is produced at discreet levels, while low-affinity epitopes, also called subdominant epitopes, only induce tolerance if the protein is synthesized at a higher rate. Subdominant epitopes present on proteins that, under physiological condition, are expressed in low amounts become so-called cryptic epitopes, a functional term that describes sequences that fail to reach the threshold to induce tolerance [49, 82]. Such cryptic epitopes can turn into subdominant epitopes on cancer cells upon over-expression of the corresponding protein, thus potentially providing targets for T cell-mediated immune responses. Devising vaccines that induce potent immune responses against such subdominant epitopes remains a challenge. Easier by far, at least with regard to the choice of antigens, is the design of vaccines to virally induced cancers. A number of viruses, such as herpes viruses[26] and papillomaviruses[5, 108], contribute to cell transformation, and expression of viral antigens is causatively linked to uncontrolled cell growth. Such viral antigens, which are foreign to the host's immune system, provide ideal choices for vaccines to the corresponding malignancies. Nevertheless, patients that develop cancer upon infection with these viruses are genetically most likely low responders to the viral antigens, thus again necessitating the design of vaccines that induce responses to subdominant epitopes.

Another challenge encountered by tumor vaccinologists is the uncanny ability of tumor cells to evade immune surveillance. Pathogens such as viruses have evolved mechanisms to subvert immune responses [53]. Vaccines to infectious agents are generally given prophylactically, thus circumventing many of these problems. Tumor vaccines are designed for therapeutic treatment of cancers. During the time interval between the first uncontrolled cell division and the onset of clinical symptoms, which can span several years, tumor cells evolve pathways not only to dodge immune surveillance[81], but also to actively subvert the immune system, thus antagonizing active immunotherapy[54].

Another question that remains to be addressed before we can intelligently design tumor vaccines is what type of immune responses are most suited to eradicate tumor cells. This will depend largely on the type of cancer as well as on the tumor-associated antigen chosen as immunogen. Tumor-associated antigens expressed on the cell surface, such as B and T cell idiotypes on certain forms of lymphomas, might provide targets for antibody-mediated cell destruction[89]; intracellular tumor-associated antigens remain elusive to antibodies, but might provide targets for T cells. Cytolytic $CD8^+$ T cells have been assumed to play a major role in tumor cell eradication; however, many types of tumor cells down-regulate proteins that are involved in the MHC class I-processing pathway, thus evading recognition by $CD8^+$ T cells[28]. $CD4^+$ T cells recognize antigen in association with MHC class II determinants.

MHC class II molecules, unlike MHC class I determinants, are only expressed on a few specialized cells but not on most tumor cells. $CD4^+$ lymphocytes are now being appreciated for their ability to limit the growth of tumor cells [63], presumably by indirect recruitment of inflammatory cells, such as eosinophils, or by secretion of tumoricidal cytokines.

A number of different types of tumor vaccines have been designed and tested, first in experimental animals and, where pre-clinical results were of sufficient promise, in clinical trials. The initial tumor vaccines were based on irradiated tumor cells, an approach that has been refined by additional modifications of such tumor cells, for example by *in vitro* introduction of genes encoding cytokines or co-stimulatory factors[62] or by introducing tumor antigens into professional antigen-presenting cells (APCs)[68]. This approach has the advantage of offering all of the potential tumor-associated antigens to the immune system, but the disadvantage that the number of tumor cells that need to be generated for this vaccine strategy are often not available. Furthermore, this vaccine regiment requires individualized vaccines for each patient. As an alternative, subunit vaccines based on single tumor-associated antigens, or even epitopes, can be developed and used for a broader spectrum of patients. Different carriers that selectively favor induction of distinct immune responses can be used to deliver such subunit vaccines. Purified proteins in adjuvant induce antibody and $CD4^+$ T cells, but are poor stimulators of $CD8^+$ T cells. Peptides in lipid formulations can induce both T cell subsets, but the response is not only monospecific and, thus, easily circumvented by rapidly mutating tumor cells, but it is also under Ir-gene control, a problem for the use of peptide vaccines in an out-bred population. Viral recombinant vaccines induce both T and B cell-mediated immune responses and are currently at the forefront of most tumor vaccine efforts.

A relatively new and promising strategy that is being exploited for both prophylactic and therapeutic treatment of infectious diseases, autoimmunity and cancer is based on plasmid DNA. Such DNA vaccines, also called polynucleotide vaccines or genetic vaccines, are circular pieces of double stranded DNA that contain sequences for selective propagation in bacteria, as well as a mammalian expression cassette composed of a constituitively active promoter, followed by a gene expressing a pathogen-derived protein or a tumor-associated antigen. Such vaccines, first described in 1992[88], have shown efficacy in animal models to a variety of infectious agents, including viruses, bacteria and parasites[47]. Vaccination utilizing naked plasmid DNA expressing tumor-specific antigens has also been shown, at least in experimental animals, to elicit long-lasting protective immune responses to malignancies [7, 16, 17, 25, 43, 77, 102].

In this review of current advances in genetic immunization, we will discuss the potential use of DNA vaccines for the treatment of cancer and, furthermore, describe avenues to enhance their immunogenicity.

Cancer Vaccines Against Model Tumors

A number of studies have tested the efficacy of anti-cancer DNA vaccines based on either model antigens or tumor-associated antigens (Table 2).

A report by IWASAKI and BARBER[39] describes a 9-amino-acid epitope from the nucleoprotein (NP) of influenza virus utilized as a surrogate tumor-specific antigen. A stable cell line was established by transfecting a DNA construct encoding the minimal NP epitope into the mouse mastocytoma cell line P815. Mice that were primed and boosted with a full-length NP construct successfully mounted a $CD8^+$ T cell-mediated antitumor response. Interestingly, protected mice challenged with the parent none-NP-expressing cell line remained resistant to tumor formation, indicating that the tumor challenge had broadened the immune response to additional antigens.

A second example utilized a construct expressing ß-galactosidase as a simulated tumor-specific antigen. Gene-gun-mediated DNA vaccination successfully protected mice from challenge by tumors expressing this gene product[72]. The vaccine was so efficacious that tumors were rejected even if DNA inoculation was given post-challenge, when tumors were already established.

To examine a potential therapy for colorectal carcinoma, an immunization strategy was developed utilizing the carcinoembryonic (CEA) gene expressed under the control of the cytomegalovirus (CMV) promoter[16, 86]. Both pig-tailed macaques and out-bred dogs were used to test the CEA vaccine. In the dogs, intramuscular, as opposed to intravenous or intradermal, injection induced the most prominent antibody titers. In macaques, intramuscular vaccination was more successful than particle bombardment. Both dogs and macaques showed significant CEA-specific lympho-proliferative responses in the absence of toxicity or histopathological abnormalities.

Lastly, a strong antibody- and $CD8^+$ T cell-mediated cytolytic response could be generated in mice primed with a plasmid vector expressing the prostate-specific antigen (PSA)[44]. In addition, this DNA-based

Table 2. Genes encoding target antigens utilized in DNA tumor vaccines

Antigen	Reference
gp75/TRP-1	25, 92, 96, 101
TRP-2	91
pmel-17/gp100	59, 92
MAGE-3	92
MART-1/MelanA	92
M-MuLV env/gag	56
scFv	34
T-ag	7, 63, 77
erbB-2/her-2/neu	1, 10, 102
CEA	16, 86
HBsAg[a]	16
p53	14, 37, 92
CRPV E1, E2, E6, E7	33
HPV-16 E6, E7	84, 92, 91
CD4	99
β-galatosidase	72
ovalbumin	72
V_H and V_L region	87, 89
$C_\gamma 1$ and C_κ region	87
Influenza NP	39, 58
HSV glycoprotein B	25
LCMV NP	71
PSA	44
HuD	60
hCGβ	30, 50

M-MuLV – Moloney-murine leukemia virus, scFv – Bcl-1 lymphoma surface Ig, T-ag – SV40 large T antigen, erbB-2 – receptor tyrosine kinase of the ErbB growth factor receptor family, CEA – carcinoembryonic antigen, HBsAg – hepatitis B surface antigen, CRPV – cottontail rabbit papillomavirus, HPV-16 – human papillomavirus type 16, V_H and V_L – human variable Ig genes, $C_{\gamma 1}$ and C_κ – human constant Ig genes, HSV – herpes simplex virus, LCMV NP – lymphocytic choriomengitis virus nucleoprotein, PSA – prostate-specific antigen, HuD – paraneoplastic encephalomyelitis antigen, and hCGβ-beta-chain of human chorionic gonadotropin.

[a] CEA and HBsAg on single plasmid.

vaccine also generated a significant proliferative T cell response.

Immune tolerance may prevent immunity directed against self-antigens. For example, the tyrosine family proteins are differentiation antigens that are recognized in patients with melanoma[36]. WEBER et al.[101] have investigated whether tolerance can be broken by priming with an xenogenic antigen. They immunized mice with a DNA construct expressing the human brown locus protein (gp75/tyrosine-related protein-1). They found that the human gp75, but not mouse gp75, elicited an immune response that provided significant protection against a tumor expressing the mouse homologue. Protection was mediated by $CD4^+$ and $NK1.1^+$ cells.

Similar to p53, ErbB-2 is over-expressed in several human cancers[93]. Both humoral and cellular immunity to ErbB-2 have been observed in cancer patients[23]. To examine the potential of ErbB-2 as a target for immunotherapy of cancer WEI et al.[102] generated ErbB-2 constructs encoding full-length or mutant ErbB-2. One construct expressed a full-length mutated ErbB-2 (ErbB-2A). This mutant eliminated the tyrosine kinase activity of ErbB-2, which is involved in cell transformation. The second construct utilized the full-length ErbB-2 with the endoplasmic reticulum (ER) signal sequence deleted (cyt ErbB-2), thereby delivering the ErbB-2 antigen to the cytoplasm for entry into the MHC class I pathway. The final DNA construct contained both of the above-described mutations (cyt ErbB-2A). Only the vaccines expressing the non-mutated wild-type ErbB-2 and the transformation-defective ErbB-2 (ErbB-2A) induced an immune response that inhibited growth of ErbB-2-positive tumors in mice. The lack of efficacy of the cyt ErbB-2- and cyt ErbB-2A-expressing vaccines would suggest that tumor-specific immunity in this model requires activation of $CD4^+$ T cells.

Idiotypes on certain types of lymphomas provide well-defined target antigens for immune intervention. Protein vaccines, especially when fused to cytokines, have been shown previously to induce protective immunity to such types of malignancies[89]. Unmodified idiotypes expressed by DNA vaccines failed to induce immune responses. These could be rescued by expressing the idiotypes as fusion proteins linked either to a carrier, such as KLH or tetanus toxoid, or to cytokines or chemokines[3].

The replication-defective Moloney-murine sarcoma virus (M-MSV) requires the helper function of the Moloney-murine leukemia virus (M-MuLV) for replication. The combination of the two viruses in the murine host allows the formation of sarcomas at the site of infection. MILAN et al.[56] generated DNA-based vaccines that express either the gag or env structural proteins of M-MuLV. Both plasmids were capable of eliciting strong cytotoxic T lymphocyte (CTL) responses and provided protection against subsequent tumor challenge.

Recent experiments in the area of cervical cancer have been performed using a cottontail rabbit papillomavirus (CRPV) model. Rabbits were vaccinated with individual early genes of CRPV or combinations of early genes by gene gun-based intracutaneous vaccination. A combination of the E1, E2, E6 and E7 genes protected over 75% of challenged rabbits[33].

An attractive target for an anti-tumor vaccine is the tumor-suppressor gene product p53. Intradermal and i.m. immunization of mice with a human wild-type p53 DNA construct provided complete protection from

challenge with human mutant p53-transfected syngeneic mouse tumor cells[37].

These experimental examples indicate that DNA vaccines can induce protective immunity to tumor challenge or even cause regression of already established tumors. Similar studies have been published for an array of other vaccines, including peptides, purified proteins, anti-idiotypes or viral recombinant vaccines thus posing the question if DNA vaccines might really be superior to other vaccines, which in clinical trials often gave disappointing results.

The Advantages of DNA Vaccines to Cancer

DNA vaccines, as they cause *de novo* synthesis of antigen in transfected cells, induce a full spectrum of immune responses, including antibodies, $CD4^+$ and $CD8^+$ T cells, all of which might limit the spread of a given tumor. This is also achieved by other vaccines, most notably viral recombinants. DNA vaccines provide their own adjuvants in the form of bacterial unmethylated CpG sequences present in the vector backbone which initiate secretion of chemokines and cytokines, such as IL-12[46], which generally drive the immune response towards the Th1 pathway and might, potentially, override the tumors' subversion of the immune system towards the Th2 pathway. Although viral recombinants do not require adjuvants to initiate immune responses, their propensity towards Th1 responses is less clear-cut. DNA vaccines are safe, unlike viral recombinants they pose no risk to immuno-compromised patients. Cancer is predominantly a disease of the elderly, a population that responds poorly to traditional vaccines. The efficacy of DNA vaccines to induce immune responses in the aged remains to be tested. DNA vaccines are easy to construct. Once the cDNA encoding the wanted immunogen is available, a DNA vaccine can be custom made in less than a week. In comparison, generation of individualized recombinant vaccines or tumor cell vaccines takes far longer.

Potentially the biggest advantage of DNA vaccines over more traditional vaccines is their apparent ability to induce responses to subdominant or even cryptic epitopes. This has been demonstrated thus far in viral and parasite models, where DNA vaccines were shown to induce immune responses in mouse strains that were non-responsive to traditional antigens[24, 48, 76]. As detailed above tumor-associated antigens are often self proteins that have tolerized the immune system to the available immuno-dominant epitopes. A vaccine that induces an immune response to subdominant epitopes of tumor-associated antigens without causing autoimmune disease would thus be highly desirable. It is unclear how DNA vaccines induce immune responses to low-affinity epitopes. The amount of antigen expressed by DNA vaccines *in vivo* especially on APCs, is low[67]. This is compensated by the longevity of antigen expression that, depending on the antigen and the delivery of the vector, can exceed a month[21]. The combination of the strong adjuvanticity of DNA vaccines combined with the long-lasting antigen expression might favor activation of lymphocytes to rare epitopes.

A potential disadvantage of DNA vaccines for delivery of tumor-associated antigens is that many of these antigens have biological functions that contribute to cell transformation. As DNA vaccines persist *in vivo* for a prolonged time, expression of oncoproteins might pose a risk for the development of an additional malignancy. The probability of such an event is minute; a number of events are needed to cause transformation and, although a single oncoprotein might destabilize the equilibrium of a cell and thus trigger other transforming events, DNA vaccines mainly target differentiated cells, such as muscle cells or keratinocytes, that are not readily destabilized. Furthermore, DNA-transfected cells are eliminated within 1–2 months by the immune system. As a safe-guard DNA vaccine-encoded tumor-associated antigens can be mutated to lose their biological functions. For example, p53, a protein that is mutated or over-expressed in more than 50% of human cancers, can be mutated within the tetramerization domain, thus creating monomeric proteins that fail to bind to DNA[100].

Another, less easily circumvented, disadvantage of DNA vaccines is that the immune response to DNA vaccines is comparatively weak. A number of strategies are currently being tested to improve the immune responses to DNA vaccines.

Avenues to Enhance the Potency of DNA Vaccines

The immune response to DNA vaccines can be enhanced by modifying the vector or the delivery method, thus increasing uptake of DNA, transcription and translation of the transgene, and processing of the transgene product. The immune response can further be modified by including adjuvants or prime boost regimens into the vaccine formula.

The magnitude of the immune response to any

antigen, both during the acute effector phase and during long-term memory, is directly correlated to the amount of antigen presented in a suitable form to the immune system, i.e. soluble antigen for activation of B cells and fragmented antigen presented on MHC determinant by professional APCs for stimulation of $CD4^+$ and $CD8^+$ T cells. The level of appropriately expressed antigen upon DNA vaccination depends on a number of parameters, including: the amount of DNA used for priming, the design of the vector (expression cassette) and compartmental targeting of the expressed antigen within the transfected cell for optimal immune stimulation.

The amount of DNA that transfects cells upon inoculation can be optimized. The immune response is initiated by antigen expressed by professional APCs most notably dendritic cells that, upon inoculation of the vaccine, become activated and migrate to draining lymph nodes, where stimulation of T and B cells is initiated. Antigen expressed by other non-professional APCs, such as muscle cells, might contribute upon antigen-reprocessing by APCs to the induction of the immune response, although available evidence gained by so-called Van Gough experiments, in which the inoculation site was ablated shortly after DNA vaccination, indicate that this pathway plays a secondary role[90]. The rate of overall transfection can be optimized by using ballistic devises, such as gene guns[88], that shoot DNA coated to gold beads directly into the skin. This allows a dramatic reduction of the amount of DNA needed to initiate a response, but, as the amount of DNA that can be delivered this way is limited, gene gun delivery has thus far not translated into an overly impressive increase of the resulting immune response. Cationic lipids can increase uptake of DNA[31, 38, 74]. For oral delivery, DNA incorporated into polyethylene glycol (PEG) particles is not only protected from destruction during passage through the alimentary tract, but is also taken up more readily by M cells[9]. Selective targeting of dendritic cells might potentially be achieved by coating vector DNA with peptides that specifically bind to receptors expressed on APCs[22].

Once the DNA vaccine reaches the nucleus of transfected cells, the design of the vector can influence the level of antigen expression both at the transcriptional and translational level and thus, in turn, affect the magnitude of the resulting immune response. Most DNA vaccines utilize the human cytomegalovirus (HCMV) immediate early promoter with the objective to induce high levels of gene expression in all of the transfected cells. Modifying promoter spacing, addition of Kozak sequences or basic transcription factor binding sites can augment the level of transcription. For viral sequences such as those derived from human papillomavirus, optimizing codon usage for the human cell machinery can further augment transcription by avoiding the rate limitation of tRNAs for rare codons[96].

The amount of protein that reaches the compartment needed for optimal activation of B or T cell responses can be enhanced. For induction of an antibody response, the antigen has to be targeted towards the secretory pathway to reach the extracellular domain. For stimulation of cytolytic T cell responses, antigen fragments need to be targeted towards the ER for association with MHC class I determinants, while activation of $CD4^+$ T helper cells requires presence of antigen in the lysosomal compartment for binding to MHC class II determinants. Examples of genes that are being targeted as tumor specific antigens in cervical cancer are the E6 and E7 genes of human papillomavirus (HPV). These two oncoproteins are sequestered within the nucleus of HPV-infected cells. It is within the nucleus that the two viral proteins (E6 and E7) have their tumorigenic effect by associating with the two cell cycle regulators p53 and the retinoblastoma gene product, respectively. In order to generate a potent immune response to these proteins, it is necessary to relocate them from their natural cellular location to a cell compartment that allows fragmentation and presentation of the antigens with MHC determinants for presentation to T cells. This can be achieved by generating tumor antigen chimeras. Essentially, the inoculated cell expresses the tumor antigen as a fusion protein. Leader sequences[14], ubiquitin[27, 71] and the lysosome-associated membrane antigen (LAMP-1)[52] are potential alterations that override the nuclear localization domains of proteins. Leader sequences derived from viral or cellular antigens guide the protein into the secretory pathway. Proteins that fail to fold appropriately for secretion through the ER are then translocated back into the cytoplasm, where they become ubiquinated. They are then degraded by cellular proteosomes into small peptides, which are moved by specialized transporter systems, called TAP-1 and TAP-2, back into the ER. There, some of them associate with MHC class I determinants. Ubiquination of a protein leads directly to cytoplasmic degradation and translocation of peptides into the ER, thus favoring association of epitopic peptides with MHC class I determinants and, hence, activation of cytolytic T cells. Addition of LAMP sequences guides a protein towards the lysosomal pathway for association of degradation products with MHC class II determinants needed for activation of $CD4^+$ T helper cells. Several of these avenues have been tested to optimize immune responses to tumor-associated antigens. For

example, CIERNIK et al.[14] used a minigene coding for a single epitope derived from mutant p53 and demonstrated that particle gene gun-mediated DNA transfer into the skin of mice induced cytolytic T cell responses. The tumor-specific response could be enhanced if the DNA vaccine was fused with the adenovirus E3 leader sequence, targeting the vaccine to the ER. A vaccinia virus recombinant expressing the E7 of HPV-16 in its native form was shown in mice to lack efficacy against a tumor cell line expressing the same oncoprotein; in contrast, a vaccinia virus recombinant expressing the E7 linked to LAMP not only provided protection to subsequent tumor challenge, but also initiated the resolution of small pre-existing tumors[52]. The increased efficacy of the vaccine correlated both with an augmented $CD4^+$ T cell response, as predicted, as well as an unexpectedly increased $CD8^+$ T cell response.

Another avenue to enhance the immune response by the re-targeting of an antigen has not yet been tested in a tumor model; this approach is based on a fusion molecule composed of the antigen linked to L-selectin. This strategy, which is only suitable for secreted proteins, guides the fusion product towards receptors expressed on dendritic cells where, upon protein uptake, the antigen is presented very efficiently to naive lymphocytes[6].

Avenues to Enhance the Immunogenicity of DNA Cancer Vaccines

The efficacy of many vaccines can be boosted by the co-administration of adjuvants. While many adjuvants are available experimentally, few have received approval for clinical use. Recently, molecular adjuvants with more clearly defined mechanisms of action have been described. These include cytokines (GM-CSF and IL-12) and accessory molecules (CD80 and CD86).

Studies of tumor-specific immunity indicate a critical role for GM-CSF in inducing protective responses. An idiotype/GM-CSF fusion protein has been investigated as a vaccine for B cell lymphoma[87, 89]. By fusing a tumor-derived idiotype to GM-CSF, it was converted into strong immunogen capable of inducing idiotype--specific antibodies without other carrier proteins or adjuvants. The fusion protein immunization was capable of protecting recipient animals from challenge with an otherwise lethal dose of tumor cells. This indicates the potency of GM-CSF as an immune stimulator allowing development of tumor-specific immunity. In addition, co-inoculation of the GM-CSF gene with a rabies virus nucleic acid vaccine has been demonstrated to boost immune responses specifically against rabies virus, including helper T cell and antibody responses[105], studies that were subsequently confirmed in numerous other systems[47]. Thus, there is ample experimental evidence for the use of GM-CSF as a molecular adjuvant for vaccines and in inducing protective *in vivo* responses.

A number of additional cytokines, chemokines and ligands have been tested as adjuvants for DNA vaccines, as has been reviewed previously[47]. The consensus from the studies is that many factors have an effect which somewhat depends on the vaccine construct, the antigen, as well as the genetic make-up of the host. Factors that affect APCs, such as GM-CSF and CD40 ligand, consistently enhanced the efficacy of DNA vaccines[42, 55, 103], while other cytokines, such as IL-2, IL-4, IL-12 and IFN-γ, gave highly variable results[2, 12, 13, 43].

An important feature of nucleic acid vaccines is that unmethylated CpG dinucleotides found in the DNA of bacterial vectors have inherent adjuvanticity for the mammalian immune system by activating B cells, macrophages and other bone marrow-derived APCs[46, 75, 106]. Through mechanisms yet to be elucidated, unmethylated CpG sequences directly stimulate macrophages to produce pro-inflammatory cytokines such as IL-12[8]. The proinflammatory effects of unmethylated CpG sequences in nucleic acid vaccines are likely to be critical for their capacity to induce immunity. Attempts to further improve cDNA vaccine by incorporating additional CpG sequences into the vector backbone have been fairly unsuccessful[64], which might reflect a delicate balance between the positive effects of CpG-induced cytokines on activation of a primary immune response on one hand and a negative effect of such cytokine on transcription driven by viral promoters on the other hand.

DNA vaccines, although by themselves often not overly efficacious, seem to be particularly suited to prime the immune response to further vaccination with more traditional vaccines such as proteins or viral recombinants. This approach, which has given promising results in several microbial systems[61, 69, 78, 80], remains to be tested systematically for cancer vaccines.

Conclusions

This review illustrates how DNA-based vaccines might be highly suitable for the treatment of cancer. The ease with which the DNA vaccines can be generated and produced, as opposed to live, attenuated or recombinant vaccines, is complemented by their ability

to generate prolonged intracellular gene expression. This continuing priming of the immune system produces long-lasting immunity[7] and the endogenous expression of antigen and presentation by MHC class I molecules provides stimulation of anti-tumor cytolytic T cell responses.

Several studies described in this review have demonstrated the ability of DNA vaccines to induce both humoral and cell-mediated immunity specific for certain tumor-associated antigens. Furthermore, DNA vaccines encoding cytokine genes or MHC class I genes can stimulate local immune responses through the recruitment of immune cells to the tumor site. Complete efficiency of tumor regression has not been displayed in the few clinical trials that have been carried out, necessitating further optimization of these vaccines.

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