Review

Current Concepts in Cancer Vaccine Strategies

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strategy for future vaccine development.

INTRODUCTION

The application of immunotherapeutic principles to the treatment and prevention of cancer has a rich heritage (2,88). The recent definition of tumor-specific immunity in cancer patients and the identification of tumor-associated antigens (TAA) have generated renewed enthusiasm for the application of immune-based therapies to the treatment of malignancies (21,38,85,119,149). Recent developments in cancer vaccines have also been based on an improved understanding of the cellular interactions required to induce a specific antitumor immune response (12,15,30,71,85,142). Consequently, a number of cancer vaccine strategies have entered clinical testing (24,40). The breakneck pace at which formulations are being tested precludes a thorough review of all strategies. Subsequently, this brief review touches upon ideas being pursued with representative examples for illustrative purposes in defining current perspectives and future strategies for active specific immunotherapy of cancers.

OVERVIEW OF CANCER VACCINE STRATEGIES

There are many ways to treat cancer. In general, immunotherapy can be considered either nonspecific, such as a general immunomodulator (e.g., a cytokine), or tumor-specific (e.g., a vaccine that targets tumor antigens). Table 1 defines representative approaches to developing cancer vaccines. Early concepts were initially based on nonspecific approaches using as examples

bacterial agents that included bacillus Calmette-Guérin (BCG). Clinical trials in bladder cancer therapy evaluating these nonspecific agents or their products have proved BCG in particular to be promising for promoting a superior reduction in tumor recurrence compared with chemotherapy (86,98).

Since the nature of TAAs was unknown for a long time, many of the initial clinical studies of specific tumor vaccines involved using whole tumor cells as a source of TAAs (39,108,168). Melanoma is the most immunogenic solid tumor in humans and, as such, has served as the major model for tumor vaccine investigation in both the laboratory and the clinic (26,57). The most extensively studied melanoma vaccines in clinical trials are whole-cell preparations or cell lysates that contain multiple antigens capable of stimulating an immune response. Unfortunately, in the majority of studies, immune responses to these vaccines have not translated into a survival advantage (57).

Advances in tumor cell immunology have led to the identification of candidate tumor cell antigens that represent a diverse array of structures associated with tumor cells or tumor rejection antigen (122). This, in turn, has allowed for refinements in vaccine design. However, the exact tumor antigens that should be targeted with a specific vaccine are unknown. As current concepts have been directed toward the induction of cellular immunity, cytotoxic T cell responses (CTL), peptides derived from human tumor antigens, have been used in particular in a number of clinical trials to induce specific immune responses against autologous tumor in cancer patients (112). Optimization of tumor T cell reactive peptides (35) might be determined by considering interactions of T

ABSTRACT

Cancer vaccines are entering a new phase of popularity, in part because of the recognition of when a therapeutic vaccine is most effective and the identification of appropriate target antigens. New technologies, most notably gene transfection into dendritic cell and DNA vaccination approaches, have spurred further clinical evaluations. While many researchers consider humoral responses as not being viable for large tumors, these responses may play a role in regulating micrometastases (i.e., adjuvant setting). The recent approval of antibodies as therapeutics for cancer treatment has lent to the viability of this therapy concept. The success of carbohydrate-conjugate vaccines in bacterial systems has also renewed interest in developing such vaccines for cancer immunotherapy. Carbohydrates can be further converted into peptide/protein mimetics with several of these mimetics in clinical trials. These mimetic forms can be manipulated into DNA vaccine types that may be combined into DNA cassettes that contain CTL-associated epitopes to further define a novel

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Table 1. Representative Vaccine Types Explored for Cancer Immunotherapy

Vaccine	Antigen	Adjuvant/Carrier	Cancers	References
<u>Nonspecific Approaches</u>				
Whole allogenic or autologous tumor cells	unknown	BCG, Complete Freund's adjuvant, <i>C. Parvum</i> , dendritic cells	colon, lung melanoma, renal	85,108,168
Viral oncolysates of allogenic or autologous tumor cells	unknown	Vaccinia virus, Newcastle disease virus	melanoma, ovarian colorectal	18,47,70,148
Tumor cell extracts	unknown	Detox, Antigen presenting cells	melanoma, lung	42,107,111
<u>Tumor-Specific Approaches</u>				
Anti-idiotype	B-cell idiotype, proteoglycans, P97, gangliosides	KLH	melanoma, colon, ovarian, breast, lymphoma, prostate	17,45,46,52, 169
Purified or recombinant antigens and their epitopes as peptide or DNA	gangliosides, MART-1, gp100, TRP-1, TRP-2, MAGE-1, MAGE-2, ESO-1, Tyrosinase, Neu, P53, MUC1, CEA, HSP	KLH, Detox, QS-21, dendritic cells, Vaccinia virus, BCG, plasmids (conjugated-T cell epitopes)	melanoma carcinoma lymphoma, prostate colon, breast	16,27,72,76,87, 89,91,125,162, 171
Synthetic carbohydrate antigens	TF, Tn, sialyl-Tn, gangliosides, Globo H, LeY	KLH	breast, ovarian, melanoma colon, pancreatic	3,32,37,64, 95,106,132 152

cell receptors with peptide displayed on libraries (113) or sequence motifs that affect major histocompatibility complex (MHC) haplotype binding (145).

Peptide and cDNA phage display libraries can be used to determine the specificity of antibodies present in whole sera of patients where information about the parental antigen is unknown. In this respect, patient serum antibody-binding ligands have been identified (58,150,165). Such ligands would facilitate the design of diagnostic assays and therapeutic vaccines. In the case of cancer, this novel technology is expected to improve our understanding of the immune responses against tumor cells and to discriminate between autoantigen and true tumor-specific antigens.

Clinical trials with single peptides have been disappointing, and multiple peptide delivery is more encouraging, although help in the form of cytokine and dendritic cell (DC) presentation seems necessary (80,116,153). On melanoma cells, autologous protein antigens that are potential targets in-

clude Melan-A, MART-1, gp100, tyrosinase, TRP-1, and TRP-2 melanoma differentiation antigens, and MAGE-1, MAGE-3, and ESO-1 cancer testis (CT) antigens (137). Cell-mediated immunity and humoral immune reactivity have been demonstrated against autologous antigens on adenocarcinomas (i.e., breast, lung, gastric, and pancreas cancers). These antigens include CEA, HER-2/neu, MAGE-1, p53, MUC-1 (56,159), prostate-specific antigen (PSA) (69) produced by prostate cancers, and alpha-fetoprotein (AFP) produced by liver cancers (hepatomas). Immunization of cancer with anti-idiotypic antibodies mimicking a variety of tumor antigens has also seen resurgence (19,41,139).

Among autologous proteins, the use of tumor-derived heat shock/chaperone proteins (HSPs) as anticancer vaccines is gaining wider study and acceptance (62,104,170,173). Heat shock proteins promote cell survival under adverse environmental conditions. HSPs have been correlated with tumor progression, promoting resistance to both che-

mical- and radiation-induced apoptosis (50). Consequently, apoptosis remains a significant problem in the treatment of some cancers. HSP expression inhibits apoptosis as mediated by the production of survival factors such as Mcl-1, Bcl-2, Bcl-X(L), and glutathione-S-transferase (GST) (22,50).

Heat shock protein-based vaccines have been shown to immunize against cancer and infectious diseases in both prophylactic and therapeutic protocols (155). So far, four classes of HSP preparation—gp96, HSP90 (hsp86, hsp84), HSP70 (hsc70, hsp70), and calreticulin—have been used successfully in tumor models. Vaccination of inbred mice with tumor-derived stress proteins hsp70, hsp90, and gp96/grp94 elicits a protective immunity to the tumor from which the vaccine was purified (63, 167,175). Methods for purifying HSP individually are now readily available (103,155).

Pilot studies on immunization of cancer patients with HSPs are ongoing (72). Immunization with autologous gp96-elicited MHC I-restricted, tumor-

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specific CD8(+) T lymphocytes and the expansion of the NK cell population was observed in the majority of patients immunized. These observations are entirely consistent with the murine experience and form a firm basis for future trials with clinical end points, using autologous, patient-specific HSP-peptide vaccine (72).

There is now comprehensive experimental evidence that the antigenicity of tumor-derived hsp70, hsp90, and gp96 preparations results from diverse arrays of endogenous peptide antigens complexed with stress proteins (11,62). Vaccination with tumor-derived stress protein/peptide complexes leads to their uptake and processing by professional antigen-presenting cells and to presentation of associated tumor peptide antigens to cytotoxic T cells (173). This induces a tumor-specific CTL response. The attractiveness of the concept of using tumor-derived stress proteins as vaccines is derived from two observa-

tions: (i) tumor stress protein vaccines mirror the individual antigenicity of a tumor, which results from random mutations due to genetic instability; and (ii) stress proteins represent powerful adjuvants for the peptide antigens complexed to them (63). DNA vaccination using HSPs as fusion proteins is being considered as a way to greatly enhance the potency of DNA vaccines via the CD8-dependent pathway (34).

The success of bacterial vaccines inducing humoral responses that target carbohydrates on bacteria (5,25,51,117) has spurred interest in developing such vaccines against tumor-associated carbohydrate (TAC) antigens (90,92). Carbohydrate antigen targets have been identified that include the T/Tn/sTn and related carbohydrate antigens such as Lewis Y and Globo H (Figure 1) (84,95, 96,131,152,154), and gangliosides GM2, GD2, and GD3 on melanomas (90,92). Several carbohydrate-conjugate vaccines are in clinical trials, most

notably Biomira's THERATOPE sTN-KLH (KLH, keyhole limpet hemocyanin) cancer vaccine being tested in high-risk breast and ovarian patients (66). Carbohydrate antigens are perceived to induce only humoral responses; however, cellular responses have been noted with Biomira's THERATOPE in breast and ovarian patients (140).

Novel approaches to vaccine design using gene transfection of tumor cells with cytokines, co-stimulatory molecules, or MHC genes (28,80,129,177) are promising. The development of genetically modified "whole" tumor cell vaccines for cancer therapy relies on the efficient transduction and expression of genes by vectors. Therefore, approaches to develop vectors or optimize gene delivery are being examined (7,33,102,110,164). DNA immunization using TAA (8,134,157) or anti-idiotypic antibodies (17,157), and tumor gene transfection of DC (44,116,135,

Figure 1. Representative carbohydrate antigens that are being evaluated as carbohydrate-based vaccines for cancer immunotherapy. TN, TF, and LeY have been synthesized as cluster antigens conjugated to KLH for clinical testing (37). GM2 has also been coupled to KLH (32). MBr1 (Globo H) conjugates have shown an ability to induce immune responses in humans (152).

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138,161) or with idiotypic fragments or peptide (163) are promising.

Concepts associated with cross-priming that focuses on the essential role of CD4⁺ Th cell-activation for optimal CD8⁺ T cell-mediated killing of autologous tumors (12,15,61,114) also provide the basis for the design of novel protocols in cellular adoptive immunotherapy of cancer, utilizing synthetic peptides capable of inducing T cell help *in vivo*. While CTL are likely to participate prominently in many models of tumor rejection, indirect mechanisms of recognition/rejection have the theoretical advantage of remaining operative even when individual tumor cells evade direct contact by down-regulating MHC and/or antigen expression (36,120).

LIMITATIONS OF IMMUNOTHERAPY

Antibody- and immune T cell-mediated immune reactions to tumor cells do not always correlate with clinical response. While murine models display promise in vaccine studies, clinical trials in humans have been somewhat disappointing (20). It is becoming generally acknowledged that the use of cancer vaccines, at present, is destined to remain limited to their employment as adjuvant to traditional therapy (23) and in the management of minimal residual disease following surgical resection of the primary cancer mass (20). As therapeutic strategies to prevent development of metastases have potential impact on cancer mortality, development of these therapies requires a better understanding of the biology and molecular events of the metastatic process (31).

Several scenarios have been proposed to be responsible for tumor immune-escape mechanisms (99). There is accumulating evidence for escape mechanisms of tumor cells that include loss of antigen or class I expression, production of suppressive cytokine by tumor cells, and expression of HSP, Fas ligand, and on tumor cells. Considering the fact that escape variants expanded again after nearly complete rejection, it is important to determine how to prevent these tumor escape mechanisms to obtain durable remissions.

The elucidation of the immune deficit against cancer progression has been a difficult task with no single mechanism explaining the complicated cancer-host immune interactions. In the first instance, the failure of cancer vaccines to fulfill their promise might be due to the very relationship between host and tumor—through a natural selection process. The host leads to the selective enrichment of clones of highly aggressive neoplastically transformed cells, which apparently are so dedifferentiated that they no longer express tumor target specific molecules (123). Specific activation of the immune system in such cases only leads to lysis of the remaining cells expressing the particular TAAs in the context of the particular human leukocyte antigen (HLA) subclass and the necessary costimulatory molecules (6,99). The most dangerous clones of tumor cells, however, lack these features, and, thus, the cancer vaccine is of little use.

There is always the possibility of faulty antigen presentation, which could result in tolerance induction to the antigens contained within the vaccine and subsequent rapid tumor progression. Even if an appropriate antigen-specific response is induced, it may not be long lasting because of the absence of poor induction of a memory response (41). It has been noted that effective vaccines need to be given regularly (e.g., monthly) in the presence of even minimal residual disease (41). This may likely be due to tumor antigens being self-antigens. Although it is possible to break tolerance, induction of long lasting memory T cells may lead to autoimmune responses (147,176).

Awareness of these mechanisms of immune escape will help to direct development of the next generation of tumor vaccines. Targeting unique antigens and modulating the cytokine environment likely will be critical to comprehensive vaccine systems in the future (151). In accordance with the fact that CTL induction is associated with a Th-1 type immune response, it has been shown that CD4⁺ T cells that can secrete Th1-type cytokines have a beneficial role in protection against tumor development (68,172). The shift from Th1-type to Th2-type cytokine

production is found in progressive cancer patients (121), and T cells harvested from tumor-bearing hosts produced only Th2-type cytokines when they were stimulated *in vitro* (97). In addition to these findings, Th2-type cytokines could even accelerate the experimental pulmonary metastasis of melanoma (82). In more recent studies on immunosurveillance mechanisms, it has been concluded that CD8⁺ CTL appear to be critical for causing tumor regression, but quantity of CTL alone is not sufficient (100). Rather, qualitatively different CTL that produce more IFN- γ and remain activated *in vivo* may be critical (100). Simply the Th1/Th2 balance cannot explain CD4⁺ cells that regulate the CD8⁺ response. Further studies to determine the mechanism of this regulation will be important for designing optimal immunotherapy. Concurrent abrogation of the inhibitory effects of CD4 cells without eliminating IFN- γ production may provide a successful concerted approach to cancer immunotherapy (100).

ROLE OF HUMORAL RESPONSES TO TUMOR ANTIGENS

Since the first vaccines against infectious diseases were developed, treating cancer with vaccines has been a long sought goal (38). Most bacterial vaccines function through antibody-mediated mechanisms. It is clear from past experience that all currently available treatment modalities for cancer are far from perfect (54). Clinical research in antibody-based cancer therapy has been driven for many years by the prospect of identifying cell-surface antigens with sufficiently restrictive tissue expression patterns to allow the specific targeting of antibody to tumor tissue. Few if any such antibodies capable of targeting rapidly and efficiently to solid tumors have been identified (53). Reviews of clinical trials using antibody-based therapy reveal that this approach can, in rare cases, induce complete remission in individual patients with cancer (53). Since these trials have usually involved patients with large tumor masses, tumor cell inacces-

sibility is probably a major reason for prevailing failures.

Immunoglobulins have evolved to optimally protect an organism from foreign invaders rather than to act as an efficient carrier molecule for therapeutic reagents. Antibodies that mediate cytolysis of disseminated cells are best represented by the colorectal cancer-associated 17-1A antibody, Panorex™ (approved in Germany and heading for approval in the US), which mediates tumor cell destruction through antibody-dependent effector mechanisms (14,43,83). This antibody is actually a murine antibody, bucking the present trend of using humanized antibodies in immunotherapy (55). Rituxan™ (IDEC Pharmaceuticals), the first cancer-directed antibody (humanized) approved in the US for cancer immunotherapy (B cell malignancies), also appears to mediate cytolysis through a complement mechanism (59) but might also promote apoptosis (146). In contrast, Herceptin™ (Genentech), approved for treating breast cancer, appears to mediate tumor inhibition through down-regulating the HER2 receptor (156). Active immunotherapy against TAAs may be advantageous over passive immunotherapy with MAbs by inducing sustained immunity. Consequently, cancer vaccines that induce primarily humoral responses such as carbohydrate-based vaccines will likely be most effective in the minimal disease setting, the stage when tumor cells are few and dispersed (micrometastases) (49,126,127,133).

Bacterial Model for Antitumor Response

A role played by antibodies might be viewed in the context of infectious disease systems. While tumor immunity is not the same as immunity for infectious pathogens, there are similarities. Bacterial vaccines presently approved appear to work through antibody responses, with limited evidence for cellular immunity in clearing infection (5,51,77). An important role played by B cells is also recognized in acute and chronic viral and parasitic infection (13). Vaccines against infectious disease do not prevent infection but limit its spread from its point of contact. Consequently, antibodies prevent blood-borne dissem-

ination of disease, having limited efficacy against microorganisms in tissue sites. Likewise, cancer vaccines that induce humoral responses may be effective at preventing micrometastases. Cancer patients who have been “surgically cured” (after removal of the primary cancer or positive lymph nodes) are quite similar to patients being re-exposed to infectious disease. The primary targets in both cases are circulating pathogens and the microscopic spread of tumor cells.

Tissue Rejection Model for Antitumor Response

The best argument for the potential role of antibodies in tissue or tumor rejection comes from the effects of natural antibodies against the gal (1-3) gal antigen found in primates (67). These natural antibodies, arising from exogenous expression of the carbohydrate antigen found on bacteria and food, are not just passive bystanders but are capable of inducing potent and rapid rejection of tissues in transplantation. The irony is that natural antibodies against gal (1-3) gal in primates cause rejection of pig organ xenograft. The concept of inducing autoimmune responses to self-antigens has been the focus of identifying tumor-associated self-antigens recognized by T cells as tumor rejection antigens (115,118). Only such studies have focused on the notion that CTL generation to self-antigens will ultimately reject tissue (tumor). It is apparent that both naturally occurring antibodies and T cells are present in cancer patients and the role of vaccines is to stimulate (augment or enhance) these respective immune response arms with the hope of causing autoimmune-like responses leading to tissue rejection. Unlike T cells, B cells affinity mature. Consequently, if the idea is to attack cancer, using concepts of tissue rejection, B cells are actually the most likely to target self-antigens.

CARBOHYDRATES AS SELF-ANTIGEN TUMOR TARGETS

The potential of carbohydrate vaccines in the adjuvant setting (treating minimal residual disease) suggests that

emphasis should be placed on further augmenting the immune response against these tumor antigens (178). Carbohydrate antigens are the most prominent antigen types on a tumor cell surface, being expressed at a high density. Carbohydrate antigens are generally weak immunogenic self-antigens, as they are T cell-independent antigens. Two major approaches are currently being used by various investigators to qualitatively and quantitatively modulate immune responses to carbohydrates. In the first, the carbohydrate itself coupled to carrier and/or mixed with adjuvant is used as the immunogen. In the second, protein or peptide mimetics of carbohydrates are used. Conjugation of carbohydrate to a carrier protein that elicits carrier-specific T- and B-cell responses does not necessarily enhance carbohydrate immunogenicity (101). Carbohydrate-conjugate vaccines that are in the clinic predominantly induce humoral responses that are considered beneficial because they mediate complement-dependent cytotoxicity (CDC) or antibody-dependent cytotoxicity (ADCC). IgM antibody production after vaccination better correlates with improved survival than IgG (75). It is the expectation, based on evidence from carbohydrate vaccination trials (64,75,81,91,93–95,109) that antibodies in general and anticarbohydrate antibodies in particular can play a role in vivo in tumor regression, potentially opsonizing tumor cells to prevent extravasation, intravasation, and metastasis. Anticarbohydrate antibodies have shown in animal models that they can mediate targeting micrometastases (178). Antibodies directed toward carbohydrates on T cells may also be potent T-cell activators (141).

MOLECULAR MIMICRY OF CARBOHYDRATE ANTIGENS

Some carbohydrate antigens are hard to purify or synthesize despite advances in synthetic approaches. Surrogates or mimics of carbohydrate antigens have been long proposed as possible immunogens to induce carbohydrate cross-reactive immune responses. An anti-idiotypic surrogate for the GD3 ganglioside referred to as Bec2 is being

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considered in a Phase III clinical trial along with BCG as adjuvant therapy following chemotherapy and irradiation (52). An anti-idiotypic antibody that mimics the GD2 ganglioside is also in clinical trial (46). These antibody-based vaccines may induce both humoral and cellular responses in the form of delay-type hypersensitivity (DTH) activity. Surrogate antigens may overcome unresponsiveness of patients to some carbohydrate antigens since they present their antigenic determinants in a different molecular configuration and therefore may stimulate clones unresponsive to the original antigen (143). Peptides as mimetics of carbohydrate antigens have also been proposed (4,124,128,130,160,166,174,179).

Peptide mimeotopes can induce responses targeting TAC antigens inhibiting tumor growth in vivo in a murine model (78). Peptide mimeotopes can provide immunological memory for TAC antigen (78). As T dependent (TD) antigens, they should prime for longer-lasting immune responses to TAC, providing anamnestic or secondary responses related to vaccine composition, form, and delivery. Memory or secondary responses could thwart repeated presentation of metastases by maintaining high levels of circulating antibodies.

Peptide mimeotopes may also augment cellular responses. It is possible that T cells can react with glycopeptides. It has been shown that T cells can specifically recognize synthetic glycopeptides (1,48,60,73,74,105). However, whether glycopeptides are selected for presentation through antigen processing mechanisms to eventually elicit carbohydrate-specific T cells is still an open question. Peptide mimeotopes of carbohydrate antigens have been shown to elicit cross-reactive CTLs to the MUC1 protein (9,10). However, this mimicry is more based on similarities between the peptides as opposed to T cells recognizing carbohydrate structures. Nevertheless, this observation speaks to the complexity that remains in vaccine design.

Peptide mimeotopes of carbohydrate antigens have a further advantage in that DNA vaccine strategies can be implemented to augment carbohydrate-reactive immune responses (79) much

like using TAA or anti-idiotypic antibody encoding plasmids. DNA vaccines provide a strategy to effectively induce cellular responses to TAA including Th1-associated responses that are critical in cancer vaccine efficacy (136,144). Peptide mimetics of carbohydrate antigens encoded into DNA plasmids provide an opportunity to induce a cross-reactive Th1 response to carbohydrate antigen upon mimeotope immunization (79). In recent studies, the feasibility of inducing cross-reactive responses to TAC using DNA encoding peptide mimeotopes of carbohydrate antigens was confirmed (79). DNA immunization induced a predominant IgG2a response to the synthetic TAC antigen LeY (Figure 2). Most importantly, we observed that immunization with mimeotope-encoding DNA primed for a LeY boost (Figure 3). Induced IgM after carbohydrate boost was transient and dropped quickly. We also examined the functionality of induced antiserum using a complement-dependent cytotoxic model targeting the LeY expressing human MCF7

breast tumor cell line. As shown in Table 2, the induced cytotoxicity of the sera was increased after boosting with nominal carbohydrate. Our results suggest that DNA immunization with encoded mimeotopes can be part of prime-boost strategies to improve the response to carbohydrate antigens. However, strategies to enhance the titers must be further evaluated. The encoding of peptide mimeotopes into DNA cassettes that also include identified MHC class I epitopes that induce CTLs that target tumor expressed TAA should further define a novel strategy for future vaccine development.

Another viable strategy using peptide mimeotopes may lie in the idea that peptides can associate with the CD1 molecule expressed on DC (29). CD1 molecules comprise a novel lineage of antigen-presenting molecules, distinct from MHC class I and II molecules. Unlike MHC molecules, the CD1 molecules appear to accommodate lipid, glycolipid, and peptide antigens in their hydrophobic cavity for presentation to a wide variety of T cells

Figure 2. Kinetic isotyping of antibody responses in pcDNAggi [PcDNAggi plasmid was generated to express a mimeotope and a T-helper epitope (79)] immunized mice. Representative sera from one experimental group of mice ($n = 4$) were collected and pooled from week 2 to week 7 after immunization. Pooled sera were diluted 1:50, and serum binding to LeY was assessed by anti-IgG1, IgG2a, and IgG2b antibodies. Standard deviation bars were calculated based on triplicate samples. The experiment was repeated twice more with similar results.

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Table 2. CDC Assay with Human MCF7 Cells Using Sera Collected Before Carbohydrate Boost and Two Weeks After That

Immunization	CDC (%)	
	Before Boost	After Boost
Naive	9.93	7.45
Vector	8.69	8.69
pcDNAggi	13.66 ^a	19.67 ^b

^aStatistically significant compared with vector immunized animals at $P < 0.01$, using Student's *t* test.

^bStatistically significant compared with pcDNAggi immunized animals before boost at $P < 0.025$, using Student's *t* test. PcDNAggi is explained in legend to Figure 2.

(158). CD1a+ cells are closely associated with tumor cells (65). The presence of a high number of infiltrating CD1a+ cells in malignant neoplasms has been reported to be associated with an improved prognosis, reduced tumor recurrence, and fewer metastases. It is likely that CD1a+ cells have a role in antigen capture and presentation in human tumors. Immunizing with loading DC with peptide mimetics of carbohydrate

antigens may lend to localized responses to carbohydrates as DC infiltrate into select tumors such as breast tumors.

CONCLUSION

Recent progress in defining the immunogenic epitopes of tumor antigens and in augmenting their immunogenicity, along with new information on the

mechanisms of tumor antigen presentation, has rejuvenated the field of cancer vaccines. A variety of strategies have been developed in which human tumor antigens, when presented appropriately with co-stimulatory molecules and/or with cytokines, can break the host's natural tolerance toward its tumor and induce rejection strength immune reactions, even in patients with metastatic disease. The identification and availability of tumor-associated antigens now allows the possibility of eliciting humoral (antibody-mediated) and cell-mediated immunity to be tested, which may result in direct or indirect tumor destruction. As cell-mediated immunity is considered important in cancer vaccine efforts, the identification of both MHC class I and II restricted tumor antigens provides new opportunities for the development of therapeutic strategies against cancer. Based on historical vaccine concepts, the relative importance of antibody responses to tumor antigens is also being reconsidered. TAC-based vaccines induce primarily humoral responses to tumor cells much as they do in bacterial applications. This reconsideration also provides new opportunities. The interconversion of TAC antigens into peptide forms has the potential to further manipulate immune responses to target these antigen types. Ultimately, for successful active specific immunization against human cancers, an understanding of the immunoevasive maneuvers of the tumor cell is still essential.

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Figure 3. Serum reactivity with LeY in DNA immunized mice before and after LeY boost. Immunized mice were bled and boosted with carbohydrate (8th week) and then bled again one (9th week) and three weeks (11th week) after boost. Collected sera from pooled blood were diluted 1:50 and studied for binding to LeY by ELISA using goat anti-mouse IgG as secondary antibody. PcDNAggi is explained in legend to Figure 2.

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