VACCINE THERAPY FOR CANCER – FACT OR FICTION?

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INTRODUCTION

In the 1890s, William B. Coley started to treat cancer patients with inoculations of bacterial extracts (Coley's toxins) to activate the general systemic immunity, of which some might be directed against the tumour.[1, 2] Subsequent efforts to increase our understanding of the molecular basis of immune recognition and immune regulation of cancer cells has led to the identification of putative new targets on tumour cells, and the potential to create potent and specific cancer vaccines. In this review the principles of tumour immunity, the tumour antigens that can be recognised by the immune system, the different types of vaccines that have already been evaluated, and the potential clinical applications of these approaches are discussed.

PRINCIPLES OF TUMOUR IMMUNITY

Unlike most vaccines for infectious agents, the ultimate aim of cancer vaccination is therapeutic and not prophylactic, and this can be achieved by activating immune responses against tumour antigens. The immune response can be crudely divided into either antibody responses or T-cell responses. Antibodies recognise and bind to conformational determinants on cell surface proteins, and can kill the cell by either antibody-dependent cellular cytotoxicity or complement-mediated cell lysis. Conversely, T-cells recognise small proteins presented on the cell surface on major histocompatability (MHC) antigens, and T-cell activation requires a co-stimulatory signal which is usually present on the cell surface of antigen-presenting cells.

Attempts to exploit the immune system as a therapeutic strategy in cancer treatment have to overcome the host's inability to develop effective endogenous immunity against cancer. Several mechanisms have been proposed to explain this phenomenon, including the development of tumour variants lacking certain tumour antigens,[3-5] loss of MHC expression,[6-9] downregulation of the antigen-processing mechanism[10] and also expression of inhibitory molecules which may promote escape from immune surveillance including tgfß[11] and Fas ligand.[12]

A further significant component of the mechanisms of escape from immune surveillance is the induction of tolerance of mature T-cells either by anergy or physical deletion.[13,14] The development of antigen-specific T-cell anergy appears to be an early event in the tumour-bearing host,[15] and in mice antigenic tumour cells can grow progressively in immunocompetent hosts without inducing either acute or memory T-cell responses.[16, 17] Activity of T-cells requires an antigen-specific signal delivered through the T-cell receptor with the appropriate peptide/MHC complex, but also requires a second antigen non-specific or 'co-stimulatory' signal delivered by specialised antigen-presenting cells. T-cell co-stimulatory pathways determine whether T-cell receptor complex engagement results in functional activation or clonal anergy.[18, 19] Engagement of the T-cell receptor in the absence of a co-stimulatory signal results in T-cells that fail to develop full effector function and become anergic even if both signals are presented in subsequent encounters with antigen.[20]

Cancer patients and tumour-bearing mice have impaired delayed-type hypersensitivity, decreased lymphocyte lytic function and a decreased lymphocyte proliferation response,[21] and may have diminished T-cell functions *in vitro* that correlate with specific alterations in the T-cell signal transduction pathways.[22-27] The notion is that tumours are poor stimulators of immune responses and moreover may be capable of actively inducing tolerance. The aim of cancer vaccination is therefore to breakdown tolerance or activate T-cells that have escaped tolerance.

TUMOUR ANTIGENS

The rational design of a cancer vaccine depends upon the identification of tumour antigens that can be targeted by the immune system, as well as strategies in antigen presentation to overcome tolerance. Tumour antigens can be classified into various categories based on their pattern of expression:

(a) unique tumour antigens expressed exclusively in the tumour from which they were identified;
(b) shared tumour-specific antigens which are expressed in many tumours but not in normal adult tissues; and
(c) tumour-associated differentiation antigens (TADA), i.e. antigens normally expressed in the tissue from which the tumour has arisen, but inappropriately expressed by the tumour.

Furthermore, oncogene products, tumour-suppressor gene products, and viral antigens in virus-associated tumours are also candidates for targeting by the immune system.

(a) Unique tumour antigens

True tumour antigens are uncommon in humans; those described in mice are probably provided by retroviral antigens. However, a number of antigens derived from the products of gene mutations have been identified in melanoma, including a peptide derived from a mutation in the cyclin-dependent kinase 4 gene (that can disrupt the cell-cycle regulation exerted by the tumour suppressor gene p16, INK4a),[28] and a product of a mutation in the ß-catenin gene (which may play a role in melanoma progression).[29] However, these unique antigens cannot be used as targets in the design of generic cancer vaccines.

(b) Shared tumour-specific antigens

The majority of shared tumour antigens isolated from mice and human tumours are reactivation of genes not normally expressed in adult tissues but activated in some tumours.[30] The best characterised example of shared tumour-specific antigens in humans is the MAGE gene family. Boon and colleagues cloned the tumour-specific antigen named

MAGE-1 which encodes the tumour-rejection antigen MZ2-E; this is recognised by autologous $CD8^+$ cytotoxic T lymphocytes.[31, 32] This antigen is expressed on many melanomas as well as other types of tumours, but not on normal tissues with the exception of testis.

Two other MAGE genes (MAGE-2 and MAGE-3) have been identified.[33] Given the shared and selective expression in tumours, these antigens are promising candidates for antigen-specific cancer vaccines.

(c) Tumour-associated differentiation antigens (TADA)

Although it would be expected that T-cells specific for self-antigens would be functionally tolerant, the majority of T-cells from melanoma patients recognise non-mutated peptides derived from melanocyte-specific differentiation antigens, most commonly from melanosome proteins; examples include tyrosinase, an MHC class II-restricted melanoma antigen recognised by $CD4^+$ T-cells,[34, 35] Melan A/MART1 and gp100/Pmel17.[36, 37]

(d) Oncogene and tumour suppressor gene products

Among the oncogenes most often associated with human cancers are members of the ras family of genes, which are mutated at high frequency in certain tumour types, including cancers of the thyroid, colon, pancreas, lung (non-small cell) and in acute myeloid leukaemia.[38] The ras family of genes consists of three functional genes – K-ras, N-ras and H-ras, which encode very similar proteins with molecular weights of 21,000. Mutations which constitutively activate the ras-induced signal transduction pathway occur at codons 12, 13 or 61 of ras genes,[39] and mutations of the ras protein can be recognised by antibodies and T-cells in both healthy individuals and cancer patients.[40-42]

Mutations in the p53 tumour suppressor gene are among the most common genetic alterations found in human cancers.[43, 44] Cytotoxic T-cell response can be generated against tumours with a mutant p53 protein following vaccination with a synthetic peptide designed to correspond to the MHC class 1 epitope generated from the particular p53 mutated protein.[45, 46] However, cancer vaccines aimed at a specific p53 mutation would be impractical in clinical practice as any single p53 mutation is present in a very small proportion of human cancers. Vaccination against wild-type p53 could potentially have a broader application as it would work against any tumour over-expressing p53 without accurately defining the precise mutation. Indeed, vaccination with wild-type p53 recombinants is equally effective in protecting animals against tumour challenge as is vaccination with mutant p53 recombinants.[47]

An additional oncogene that is a potential target for vaccine design is the HER2/neu proto-oncogene. Although no mutations of this gene have been found, amplification and over-expression of the gene have been demonstrated in a variety of human tumours including breast, ovarian, uterus, lung and colon cancers;[48] over-expression correlates with aggressiveness of malignancy and poor prognosis in breast and ovarian cancers.[48, 49] The HER/2/neu derived peptides can elicit a cytotoxic T lymphocyte response by primary *in vitro* immunisation in culture systems.[50] Moreover, immune manipulation of this oncogene product with a monoclonal anti-receptor antibody can effectively prevent the development of tumours in a transgenic mouse model over-expressing the rat neu oncogene in mammary epithelial cells.[51]

(e) Virus-associated tumour antigens

Specific viruses have been implicated in the aetiology of a number of human cancers, raising the possibility that viral antigens could be exploited as tumour-associated antigens for the purpose of vaccine design. The hepatitis B virus is closely associated with hepatocellular carcinoma, although the cause-and-effect relationship is unproven.[52-54] In addition to the possibilities of developing a therapeutic vaccine, prophylactic hepatitis B vaccination can be used in high risk areas as a cancer prevention strategy.[55]

The role of human papillomaviruses (HPVs) in the development of cervical carcinoma has been well documented, with HPV DNA detected in more than 90% of these tumours predominantly of the HPV16 and HPV18 genotypes. The majority of cervical cancer cells express the E6 and E7 antigen, and CTL responses have been observed *in vitro* in patients with HPV-associated cervical lesions.[56, 57] This raises the possibility of designing therapeutic vaccines against these antigens.

VACCINE DESIGN: CELL-BASED OR ANTIGEN-SPECIFIC?

Historically, the initial cancer therapeutic vaccines were cell-based, i.e. the approach was to use the tumour cells themselves as a source of antigen. Indeed, this approach has potential advantages in that the vast majority of tumour rejection antigens remain unknown. However, for this approach to be effective it must generate a stronger immune response to the tumour-associated antigens than to the expressed self-antigen within the tumour. Therefore, the development of immune tolerance during tumour development would be a potential drawback of this approach, and a further disadvantage is the poor expression of both MHC and co-stimulatory molecules by cancer cells. Early clinical studies, for example in melanoma, used vaccines consisting of tumour cells mixed with adjuvants such as BCG[58] or DETOX.[59] Subsequently, genetic modification of these cell-based vaccines has been evaluated in an attempt to overcome the disadvantages of poor MHC and co-stimulatory molecule expression by cancer cells. A number of cytokine genes can augment host anti-tumour immunity against transplanted tumour cells, including IL-1, IL-2, IL-4, γ-interferon, IL-6, IL-7, TNF-α, and GM-CSF (granulocyte-macrophage colony-stimulating factor) (reviewed in reference 60). Of these, the most potent appears to be GM-CSF[61] which is also a crucial factor in differentiation of precursors to dendritic cells, which are powerful antigen-presenting cells.[62]

A number of studies have also been reported where the gene transfer of the co-stimulatory molecule B7 results in the rejection of tumour cells expressing MHC I and MHC II,[63-65] and B7 can also prevent anergy.[66] Other co-stimulatory molecules such as B7-2 and GL-1 have also been identified.[67-69] Similar anti-tumour immune responses have been reported when HLA genes and co-stimulatory molecules are transfected into tumour cells. Presentation of TADAs by MHC molecules are necessary for immune recognition of TADAs. Non-immunogenic animal tumours which lack MHC class I expression can be rendered immunogenic when MHC expression is restored following gene transfer.[6, 7, 70-73] Similar enhanced anti-tumour immune response can occur after transfection of MHC class II molecules.[74, 75]

In several of the early studies autologous tumour cells were used as cell-based vaccines. However, this approach would be highly individualised, labour-intensive and therefore relatively impractical for use in clinical studies. Given that many tumour antigens are shared rather than unique, an alternative approach is to use an allogeneic cell-based vaccine, i.e. standard tumour cell lines derived from other patients. This has the advantages of being more practical for use in clinical practice, and, as 'foreign' material, may also amplify the immune response. Indeed, there is evidence that tumour antigens are presented by host bone marrow-derived cells rather than by the vaccinated tumour cells[76] and so MHC compatability between patient and tumour is not necessary for function of an allogeneic vaccine.

CANCER VACCINES: ANTIGEN-SPECIFIC

The notion of directing the immune response towards a selected antigen should potentially give greater control of the immune response. However, tumour-associated antigens have not been identified for most tumours, and where identified, they may not be the most potent antigens involved in the rejection of that particular tumour so that vaccine design may be sub-optimal. Peptide vaccines, viral vaccines, bacterial vaccines and nucleic DNA vaccines are all stimulatory mechanisms that can be used to activate immune responses against a specific antigen.

Most of the peptide vaccines have used MHC class I-restricted antigenic peptides. Examples include an HLA-A1-restricted MAGE-3 peptide in metastatic melanoma,[77] and an HLA-2-restricted gp100 peptide synthetic analogue, also in melanoma.[78] Indeed, 42% of 31 patients treated with this vaccine plus IL-2 had objective therapeutic responses.[78] Whether this combination is superior to IL-2 alone remains to be determined. Furthermore, although peptide vaccines require loading of MHC molecules onto antigen presenting cells (APCs) *in vivo*, administration of peptide without targeting activating APCs can potentially load MHC molecules on non-professional APCs, resulting in induction of tolerance rather than activating an immune response.[79, 80]

Genes encoding specific tumour antigens can also be introduced into the viral genome by standard techniques to create recombinant viral vaccines. Recombinant vaccinia,[81-83] adenovirus[84] and fowl pox[85] vaccines have been evaluated in pre-clinical models as cancer vaccines. Preliminary results of clinical trials with recombinant vaccinia vaccines expressing CEA[86] or HPV E6 or E7[87] in humans have shown that these vaccines can induce an immune response. Furthermore, recombinant bacterial vaccines have potential as cancer vaccines. Several bacteria including salmonella,[88, 89] BCG[90, 91] and *Listeria monocytogenes*[92] are potentially infective by the enteric route (raising the possibility of an oral vaccine), and can also target antigens to professional APCs. *Listeria monocytogenes* has the additional advantage of being able to 'live' in the cytoplasm of the cell and thus target protein antigens to the cellular arm of the immune response. Recombinant *Listeria monocytogenes* vaccines that secrete a tumour-specific antigen can protect mice against lethal challenge with colon or renal cancer cells that express the antigen, and can also induce regression of established tumours (colon and renal cancers and melanoma) in animal models by an antigen-specific T-cell dependent mechanism.[93, 94]

Naked DNA vaccines can also induce tumour antigen-specific immunity. Direct injection of plasmid DNA into mouse muscle or skin, without any transfection agent, results in the expression of the gene product and can stimulate an immune response.[95, 96] Furthermore, intramuscular injection of plasmid DNA encoding influenza A nucleoprotein in mice results in the generation of specific CTLs and protection from a subsequent challenge with a heterologous strain of influenza A virus.[97] DNA vaccines could potentially be used as immunotherapy of malignant disease by re-injection of plasmid DNA encoding tumour-specific antigens, but they have poorer efficacy than vaccination with recombinant viruses.[98] Numerous strategies have attempted to induce improved immune responses over intramuscular injection of DNA including transdermal[99] or mucosal[100] delivery, gene-gun delivery of DNA-coated gold beads[100] and DNA-liposome complexes.[101] The feasibility, safety and therapeutic potential of the latter has been demonstrated in a small study in patients with melanoma.[101] Encapsulation of plasmid DNA in poly (DL-lactide-co-glycolide) microparticles can protect plasmid DNA against degradation after oral administration, and can induce immune responses.[102] This approach has potential in developing cancer vaccines that can be administered orally.

CANCER VACCINES: POTENTIAL CLINICAL APPLICATIONS

Many early clinical studies have evaluated cancer vaccines in melanoma, although many of these clinical observations involved only a small number of patients. In an early study in patients with metastatic melanoma (n=80), treatment with vaccinia melanoma cell lysates was reported to give an improved overall survival after two years follow-up, although this was in comparison with historical controls.[103] Other studies have suggested a survival benefit in stage II patients,[104] in stage IIIA and stage IV patients treated with a polyvalent melanoma cell vaccine,[105] and in stage II patients treated with a polyvalent melanoma vaccine.[106] Furthermore, a phase II study of vaccine therapy comprised of allogeneic and autologous human melanoma cells infected with live Newcastle disease virus oncolysate in patients with stage III melanoma following therapeutic lymph node dissection gave a 55% 15-year overall survival.[107] However, none of these vaccines were evaluated in a randomised phase III study, and the survival benefit was extrapolated from comparison with historical-matched patients. The interim analysis from a phase III study in surgically resected stage II melanoma randomising to either vaccinia melanoma oncolysate vaccine or placebo vaccinia virus vaccine showed no survival advantage after a mean follow-up of 42 months, although retrospective subset analysis did suggest a significant survival benefit in favour of the vaccine in clinical stage I patients.[107]

Until these various vaccine approaches have been evaluated in randomised phase III studies against standard therapy, their activity in advanced disease or in the adjuvant setting remain speculative: the results from these phase II studies are encouraging and have confirmed the safety of this approach.

Other vaccine approaches currently undergoing early clinical evaluation in melanoma include irradiated, autologous melanoma cells transfected with IL-2,[109, 110] also well tolerated with mild systemic symptoms of fever and headache. In both the studies carried out either CTL or delayed-type hypersensitivity response was confirmed, and

although no objective response was observed, disease stabilisation was noted in three of 12 patients,[109] and in five of 15 patients[110] respectively, raising the notion that these approaches may have activity in minimal disease states. Similarly, a phase I trial of a vaccine consisting of the minimal epitope, immunodominant 9-amino acid peptide derived from the MART-1 tumour antigen in 25 patients with stage IIB-IV melanoma, showed that this approach is well tolerated.[111]

Several clinical studies have attempted to exploit the expression of CEA by colonic (and non-colonic) carcinoma cells in the design of therapeutic vaccines. A recombinant vaccinia-CEA vaccine (rV-CEA) can elicit a specific CTL response which is MHC-restricted.[112] A murine monocloncal anti-idiotype antibody, which mimics a specific epitope on CEA, was evaluated in 12 patients.[113] This study demonstrated that the vaccine was capable of breaking 'immune tolerance' to CEA in patients with CEA-positive tumours, and although toxicity was limited to mild fever and chills, all patients had disease progression after four to 13 dosages. Two phase I studies of rV-CEA, in 17 and 20 patients respectively, confirmed that the vaccine was well tolerated,[114, 115] with toxicity limited to mild local and systemic reactions comparable to those seen with vaccinia alone.[114] However, most of these patients with advanced colorectal cancer had tumour progression demonstrated by clinical and radiological assessment or by CEA levels.[114, 115]

It appears likely that cancer vaccines, like other forms of cancer immunotherapy, will have most anti-tumour impact in minimal disease states. Interestingly, a phase I study in 20 patients using an autologous tumour cell vaccine modified by Newcastle disease virus has confirmed the safety of the vaccine (mild fever in four of 20 patients) when used adjuvantly after surgical resection of the tumour.[116] It is likely to be evaluated in a large randomised clinical trial.

Encouraging results have also been noted in a randomised trial of autologous tumour cell-BCG vaccination versus observation following surgical resesction of stage II or III colon cancer, with a significantly longer recurrence-free period and recurrence-free survival with vaccination in stage II (but not stage III) disease, but with no improvement in overall survival.[117]

A phase I/II study of a recombinant vaccinia virus expressing the E6 and E7 proteins of HPV16 and 18 (TA-HPV) has been evaluated in eight patients with late stage cervical cancer.[87] No clinically significant side-effects and immune responses were observed.[87] Similarly, a phase I/II trial of a vaccine consisting of two HPV16 E7 peptides and one helper peptide, emulsified in adjuvant, in 19 patients with HPV16-positive cervical cancer refractory to conventional treatment confirmed that this was well tolerated, and stable disease for one year after vaccination was seen in two patients.[118] This strategy warrants further evaluation not only in advanced cervical cancer but also in pre-invasive malignancy.

Sialyl-TA (STn) is a carcinoma-associated core region carbohydrate antigen of epithelial mucin, and its expression is associated with a poor prognosis in colon,[119] gastric,[120] ovarian[121] and breast cancer.[122] In a phase I study in patients with metastatic breast cancer, immunisation with a synthetic STn linked to keyhole limpet haemocyanin (KLH) and given with an immunological adjuvant (DETOX-B) gave rise to the development of specific IgM and IgG antibodies in all patients, and two of the 13 patients treated in this study had a documented partial response.[123] Measurable tumour responses were also recorded using this vaccine in a randomised phase II study in patients with metastatic breast cancer. The vaccine was well tolerated apart from erythema and granuloma formation at the injection sites, and the humoral immune responses to the antigen were augmented by low-dose cyclophosphamide.[124] This vaccine is currently being evaluated in a multi-centre randomised phase III study as maintenance therapy in patients with metastatic breast cancer who have responded to chemotherapy.

Men with rising prostate-specific antigen (PSA) levels after primary therapies for prostatic carcinoma such as prostatectomy or radical radiotherapy but with no demonstrable metastatic disease represent a group of patients in whom immunotherapy, such as vaccine therapy, may be a therapeutic option. Infusions of dendritic cells, pulsed with two HLA-A2-specific prostate-specific membrane antigens (PSM-P1 and PSM-P2), at six-week intervals in 37 patients with presumed local recurrence of prostate cancer after primary treatment failure, gave one complete and ten partial responders based on National Prostate Cancer Project criteria, or on a 50% reduction in PSA, or on a significant resolution of lesions.[125] Other vaccine approaches for prostatic cancer in early clinical trials include irradiated autologous prostatic carcinoma cells transduced with GM-CSF,[126] or the complex carbohydrate molecular globo H (a candidate antigen present on prostate cancer cells) hexasaccharide conjugated to keyhole limpet haemocyanin (KLH) and administered with an adjuvant.[127] However, like melanoma, the potential benefits of vaccine therapies in the management of prostatic carcinoma need to be evaluated in phase III randomised trials.

Small cell bronchial cancer is highly responsive to chemotherapy (with or without radiotherapy) but relapses are common. Consequently, most patients die within two years of diagnosis, usually as a result of residual disease resistant to the initial therapy. Indeed, over the past two decades, no additional therapies have increased overall survival. Among the potential targets for immunotherapy identified on the cell surface of small cell lung cancer cells are the gangliosides GM2, GD2, GD3 and Fuc-GM1, as well as the carbohydrate globoH and the glycoprotein KSA. Vaccination with Fuc-GM1 conjugated to the carrier protein KLH and mixed with adjuvant is both safe and immunogenic in a phase I study in patients with small cell lung cancer.[128] This is likely to be a key component of any polyvalent vaccine against small cell lung cancer which would be worthy of further investigation as 'maintenance' therapy in small cell lung cancer after response to initial chemotherapy and/or radiotherapy with the aim of improving duration of remission, and possible overall survival.

Follicular lymphoma is associated with a characteristic t(14;18) chromosome translocation, and this can be used as a marker of minimal residual disease using a very sensitive PCR technique. Most follicular lymphoma patients in complete remission after conventional chemotherapy still have tumour cells with t(14;18) detectable by PCR. Patients with persistent circulating tumour cells seem to be at an increased risk of relapse. Idiotypic protein-KLH vaccination with GM-CSF has been evaluated in 20 patients with follicular lymphoma in a chemotherapy-induced first clinical complete remission.[129] All 11 patients with detectable

translocations in their primary tumours had cells from the malignant clone detectable in their blood by PCR, both at diagnosis and after chemotherapy, despite being in complete remission. Following vaccination no cells from the malignant clone were detectable in the blood of eight of 11 patients who also sustained their molecular remissions. Tumour-specific CTLS were also found in 19 of the 20 patients. Vaccination was thus associated with clearance of residual tumour cells from blood after chemotherapy, and long-term disease-free survival. These results provide the basis for a proposed randomised trial planned by the NCI comparing chemotherapy alone with the same chemotherapy followed by vaccination in patients with follicular lymphoma, with remission duration as the primary endpoint.

CONCLUSION

The notion that the immune system can be activated by cancer vaccines to attack and reject established tumours is a fact. Early clinical evaluation of these vaccines suggests that they are well tolerated with minimal toxicity although the optimal vaccine design has yet to be designed. Further clinical trials are required to determine the activity of cancer vaccines in:

(a) advanced disease;
(b) in the adjuvant setting to delay or prevent disease recurrence and to prolong overall survival;
(c) as maintenance therapy after chemotherapy; and
(d) as potential enhancers of the sensitivity of tumours to standard cytotoxic chemotherapy agents.

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