Amongst those, the choice of anti-metabolite(s) and schedule of administration are probably the most important variables. For example, rapidly growing tumours are less sensitive to the inhibitory effects of HU because of the higher levels of intracellular nucleotide pools [8]. The use of anti-metabolites with different biochemical effects (e.g. cytosine arabinoside, gemcitabine), singly or in combinations with HU, should be considered in order to inhibit different key DNA synthetic pathways inside the cell. Solveing *et al.* [7] have shown that the highest level of DTIC-induced DNA damage (due to adduct formation) in peripheral lymphocytes from treated patients was achieved within 5 h with daily treatment with DTIC 250 mg/m$^2$ and most of the DNA damage was repaired within 20 h. This may indicate that longer infusions of HU may be required to effectively block DNA repair synthesis after DTIC, because the duration of depletion of intracellular deoxyribonucleotides by antimetabolites may be critical in preventing cells from repairing damaged DNA. Longer infusions of HU will, however, increase the risk of toxicity to HU [13]. The use of other agents that are more active after depletion of $O^6$-alkylguanine-DNA alkyltransferase, such as fotemustine [14], should be assessed in combination with standbreak repair inhibition and DTIC, to maximise the inhibition of repair pathways.

Biochemical modulation of the activity of cytotoxic drugs remains an intriguing aspect of cancer therapy and requires further exploration. Information obtained from experimental systems may not be predictive of outcome in humans because of the difference in cell kinetics and biochemical characteristics of animal and human tissues.


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# Feature Article

# Cancer Vaccines

A.G. Dalgleish

FASHION TENDS to come full circle every decade or so. Consider tumour immunology and the attempted development of cancer 'vaccines' as treatment. Those few individuals attempting to treat human cancer with 'immunotherapy' a decade or more ago were very much marginalised by the mainstream therapists. Today they are undergoing a rehabilitation, in part made possible by the biotechnology revolution and the ability to dissect and alter the immune responses to tumours. A century ago, dogs and donkeys were used to raise sera against human tumours to treat cancer patients. Similar clinical 'trials' were to be practised for the next few decades even though regressions and clinical improvements were rarely seen (for a review, see Oettgen and Old [1]). Similar studies were revived in the 1950s and 1960s without much success. The advent of monoclonal antibodies and the ability to epitope map immune responses has not had the clinical impact that had been expected except for a few anecdotal remissions. Early studies in mice, where methylcholanthrene-induced sarcoma cells could immunise syngeneic mice so they

*Table 1. Long-standing autologous and allogeneic active specific immunotherapeutic trials in man*

| Trial | Tumour | Author minimum duration [ref] | Comments |
|---|---|---|---|
| Irradiated mixed allogeneic cell vaccine | Melanoma | Morton *et al.* [7] | Phase II increased survival in stage IIIA and IV |
| Autologous tumour cells irradiated + BCG | Colorectal | Hanna *et al.* [8] 9 years | Phase III increased survival in patients with $B_2$–$C_3$ |
| Autologous irradiated crymatically dissociated + BCG | Melanoma | Berd *et al.* [9] 3 years | > 3 cm lymph node removed. Increased survival |
| Allogeneic cell lystates + Detox adjuvant (Theracine) | Melanoma | Mitchell *et al.* [10,11] 9 years | Increased survival in advanced disease |
| Viral lysates, single allogeneic cell line (vaccinia) | Melanoma | Hersey [12] 5 years | Post lymph node resection. Improved survival |
| Viral lysate allogeneic cells | Melanoma | Wallack *et al.* [13] 10 years | Stage II? Increased survival? |
| Polyvalent soluble antigen vaccine | Melanoma | Bystryn *et al.* [6] 5 years | Stage III |
| GM2 + BCG (ganglioside) | Melanoma | Livingstone *et al.* [14] 5 years | Stage III |

became resistant to the same tumour, were to be explained by an immune response to a retrovirus of the AKR mouse. The demonstration by Lansteiner and Chase [2] that hypersensitivity to compounds could be transferred from one animal to another, led to the realisation that cellular immunity could play an important role in tumour recognition and rejection. Both major histocompatability complex (MHC)-restricted (such as cytotoxic T-lymphocytes, cytotoxic T lymphocytes (CTLs), and/or tumour-infiltrating lymphocytes, TILs) and non-MHC-restricted cells such as natural killer (NK) and, more recently, lymphokine-activated killer (LAK) cells are all recognised as having anti-tumour activity.

The recognition that the immune system could be 'pushed' or manipulated into rejecting an established tumour came with the work of Coley [3] who refined earlier observations of a number of workers, such as Fehleisen and Burns, that infectious empyemas occasionally led to resolution of a tumour (this was before the antibiotic era) and developed a collection of heat-killed bacteria (as opposed to live bacteria used by others) that produced what is now known as Coley's toxins. Detailed studies by others of the antitumour activity of these toxins were never conducted, as 'toxins' became unfashionable when the chemotherapy era began. Nevertheless, a highly refined variation on this approach using the BCG bacillus was used by a number of investigators for the treatment of solid tumours until that too became unfashionable, even though BCG was effective at inducing responses in malignant melanoma when used intralesionally although it did not affect survival as well as inducing remissions in bladder cancer when given intravesically [4].

Against this background, a dedicated band of tumour immunotherapists have persisted in trying to improve on the poor response to human vaccine approaches. The main target has been malignant melanoma [5] which is one of the few truly 'immunogenic' human cancers which responds, albeit usually transiently, and occasionally to a number of immunological manipulations including BCG, α-interferon (IFN), interleukin-2 (IL-2) and tumour vaccination strategies. The latter include using autologous and allogeneic tumour cells which have either been altered by viruses or gene transfer, or selected for their expression of tumour antigens.

There has been a tremendous effort to identify and clone tumour antigens. However, it should be appreciated that true tumour-specific antigens are uncommon in humans as those described in mice are probably provided by retroviral antigens. Human tumour antigens appear to be normal antigens inappropriately expressed which are better described as tumour-associated differentiation antigens (TADA). Many of the antigens associated with melanoma have been described, such as the gangliosides GM2, GD2 and O-acetylated GD3, as well as a lipoprotein and an oncofetal glycoprotein in addition to others. Antigens shed from melanoma cells have been used to create a melanoma vaccine for treatment and used with alum as an adjuvant in a large-scale clinical trial [6]. Variations on this theme are being pursued by a number of investigators, including those using viruses to produce oncolysates as vaccines (Table 1). As human melanomas are heterogeneous, cloning a single antigen or even a few is unlikely to result in efficacy in a large number of people. Hence, a mixture of cell lines selected for maximal expression of several melanoma antigens might be expected to induce a greater response rate. Such an approach has been followed by Morton and his colleagues [7] for nearly three decades. In their most recent study, there appears to be a survival advantage in stage IIIa and IV melanoma patients treated with a mixture of three allogeneic cell lines which express most of the known melanoma antigens, including the one recently cloned by Van der Bruggen and colleagues [15]. The survival advantage is greatest in those patients in which an immune response to the melanoma antigens can be documented. This has also been noted by several other authors including Bystryn and colleagues [6] and Hersey [12].

This work assumes greater relevance given the parallel developments in human cytokine therapy and murine tumour studies.

## LESSONS FROM ANIMAL MODELS

Non-specific immunotherapy has been actively investigated over the last few years with regard to the use of interferons and IL-2 in cancer patients. Murine models suggest that *in vitro* IL-

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2 expanded lymphocytes (LAK cells) combined with IL-2 administration *in vivo*, could cause tumours to regress that did not do so for either approach used independently. The use of LAK cells and subsequently TILs with IL-2 in tumours has been extensively reviewed elsewhere [16, 17]. Briefly, the promise suggested by the mouse model experiments was not realised in human studies, although a few long-term responses were seen in patients with melanoma and renal cell carcinoma. The toxicity associated with the early high-dose treatments coupled with the realisation that it is not the 'dose' but where the short-acting cytokine is placed, had led to the use of gene transfer into TIL cells and into tumour cells. The rationale for inducing IL-2 expression in TIL cells is to induce IL-2 expression in the most appropriate lymphocytes that target the tumour. Although ingenious, this approach has run into considerable problems with regard to the ability of the transferred gene expressing TILs to maintain expression and ability to 'home' to the tumour. A large randomised trial [18] is currently underway to address these issues. As the early enthusiastic reports of this approach have not been confirmed so far (and not for want of enormous financial backing from the NCI), it is reasonable to expect that the responses are neither marked nor long-lived. Even if this approach was to prove useful, it is inherently impractical and will be prohibitively expensive for most health care systems. A more practical approach is to induce the cytokine expression in the tumour cells so the T-cell recognition of tumour antigens is enhanced. There are a large number of animal models in which a tumour cell, modified by the introduction of a cytokine and other genes, loses its tumourigenic properties. Furthermore, tumour cells expressing cytokines used as a vaccine induce an immune response which prevents a challenge of the wild type (untransfected) tumour cell from taking. Unfortunately, this approach is less convincing in treating established tumours when the vaccine is used as a 'therapeutic', although some recent data give grounds for optimism [19].

What is remarkable about these studies is that it would appear that the defect in recognition of tumour cells appears to be at the affector, and not at the effector, arm of the immune response. In other words, that it is theoretically possible to re-programme the immune response so that it will see otherwise invisible tumour cells. Another remarkable feature of these studies is that so many cytokines and other genes are capable of inducing an effective anti-tumour response. Different mechanisms are involved with some cytokines stimulating local inflammatory responses and others a specific immune response. Some do both. Cytokines whose expression in tumour cells renders them non-immunogenic include IL-1 [19], IL-2 [20–24], IL-4 [25, 26], IL-6 [27–29], IL-7 [30–33], α-IFN [34], α-tumour necrosis factor (TNF) [35, 36], granulocyte colony-stimulating factor (G-CSF) [37] and granulocyte–macrophage CSF (GM-CSF) [38]. Other cytokines have also been reported to induce anti-tumour responses [39]. However, these models are also able to demonstrate the lack of effect on anti-tumour responses by other cytokines such as IL-5 and IL-10 [39, 40]. Whereas some tumour cells are directly inhibited by the insertion of a cytokine gene, the majority of tumour control appears to be due to the stimulation of host effector cells by the activity on the secreted cytokine. Hence some cytokines will work in some contexts [41] better than others and synergistic opportunities abound [42].

As more detailed work on the mechanisms of tumour cell killing is reported, it is clear that there are a variety of anti-tumour pathways that are recruited by different cytokines. Importantly, both local and systemic T-cell-mediated killing mechanisms are both recruited (Table 2). G-CSF induces a local inflammatory immune response whereas IL-2 and γ-IFN induce a T-cell-mediated response. Interestingly, the G-CSF transduced cell line was able to induce tumour regression [37] in contrast to most of the reported studies, although this was in an unusual tumour cell/animal model as the mice required irradiation before the tumour would 'take'. GM-CSF depends on both CD4+ and CD8+ T-cells for its activity and may enhance antigen presentation by recruiting dendritic cells which are powerful antigen-presenting cells [38].

Nevertheless, many of the mechanisms remain unclear and may manifest differently in different clinical scenarios. For example, IL-6 is able to induce tumour-specific CTL and NK, as well as have direct immunoproliferative effects in some tumour models [27], yet acts as an autocrine growth factor in some myelomas [42]. IL-7 requires CD4+ and macrophages to induce an anti-tumour response in some models and CD8+ in others [30–33]. The involvement of macrophages in some of these systems remains unclear as to whether they are assisting in the 'affector' (antigen presenting) arm or having an 'effector' tumoricidal effect.

What extent cytokine-induced inflammation has on the overall anti-tumour response is not clear. Cytokines can act directly on environmental cells such as those of the endothelium and up-regulate adhesion molecules and theoretically alter the angiogenic status of the tumour.

Most of the anti-tumour effects induced by the foregoing do not induce regression of established disease. This is clearly important for designing therapeutic strategies against human cancers. However, one or two exceptions are now being reported. IL-4 can induce a response when injected directly into a tumour

*Table 2. Mechanisms of anti-tumour immune responses with cytokine-expressing tumour cells*

| Cytokine [ref.] | Main effector cells | Other cells probably involved |
|---|---|---|
| IL-2 [20–24] | T-CD8 | NK (LAK) + PMN, Eos monocytes |
| IL-4 [25,26] | Eosinophils | CD8 monocytes |
| IL-6 [27–29] | CD4, CD8 | NK monocytes |
| IL-7 [30–33] | CD4, CD8 monocytes | |
| γ-IFN [34] | CD8 | NK |
| α-TNF [35,36] | CD4, CD8 monocytes | |
| G-CSF [37] | PMN | Monocytes, eosinophils, CD8 |
| GM-CSF [38] | CD4, CD8 | |

[39]. Cytokine-secreting tumour cells such as G-CSF-secreting cell lines already mentioned, GM-CSF-secreting B16 cell [38], IL-2-producing MBT-2 bladder cells [23] and IL-6-secreting D122 Lewis Lung cancer cells have all been reported to induce regression of established tumours or to inhibit the appearance of metastases [29]. Even though the odds are stacked heavily against the tumour cells in these experiments, these are useful models to explore the parameters required to induce meaningful responses. One factor that is clear is that an induced immune response is much more likely to be effective against a small tumour burden than a large one.

## OTHER GENES INVOLVED IN INDUCING A PROTECTIVE IMMUNE RESPONSE

Similar anti-tumour immune responses have been reported when HLA genes and co-stimulatory molecules are transfected into tumour cells. In order for the immune system to recognise TADAs, they have to be presented by MHC molecules and it, therefore, makes sense that the tumour will not be seen if MHC presentation of TADA peptides is functionally impaired. Many tumours have lower or absent MHC expression [43–46], and non-immunogenic animal tumours which lack MHC class I expression can be rendered immunogenic when MHC expression is corrected by gene transfer [47–54]. The relevance of the lack of HLA class I expression and clinical progression in some human tumours has shed doubt on the potential relevance for human therapeutic approaches. However, because humans are so MHC (HLA) heterologous, recent studies suggest that not only is the specificity of the allele important but also more than one allele may be necessary to present the full repertoire of tumour antigens to the immune system [55].

Mandelboim and colleagues [55] were able to show that in mice bearing 3LL carcinoma cells, vaccination with double MHC class I transfectants inhibited the generation of metastases whereas single MHC transfectants were only marginally effective at inhibiting the spread of disease.

As it would appear that it is the affector arm of the immune system that is defective, it is not unreasonable to suspect that MHC class II genes should also induce an effective (better?) immune response. There are technical problems though because two genes, both the α and β chain genes, of class II need to be expressed. Another possible problem is the need to have endogenous expression of the invariant chain to obtain functional assembly although the absence of the invariant chain may allow endogenous (viral?) antigens to be associated with class II and this allows an enhanced effectiveness of class II-transfected cells to present TADAs. James and colleagues [56, 57] have reported an enhanced anti-tumour immune response compared to class II transfected cells. Similar results were found in a different tumour system by Ostrand-Rosenberg and colleagues [58–60] who also showed that the positive effect of MHC class II could be negated by deleting the cytoplasmic domain [58].

## CO-STIMULATORY MOLECULES

Another way in which antigen presentation of TADAs can be rendered defective is if they do not provide the appropriate co-stimulus (in which case a presented signal results in anergy or tolerance, which could explain the failure of the immune system to see TADAs). A number of studies have now been reported whereby the gene transfer of the co-stimulatory molecule B7 (not to be confused with HLA B7) results in the rejection of tumour cells expressing MHC I and MHC II which remain tumorigenic [61–64]. Indeed, the ability to 'cure' micrometastases has also been reported. More recently, alternative co-stimulatory molecules (B7-2, GL-1) to B7 (which interact with CD28 and CTLA-4) have been identified [65–67]. Alternative pathways have been deduced to exist from experiments in CD28 -/- mice in which cytotoxic T-cells could still be induced [64]. Nevertheless, CD28 signalling is important for both the induction of IL-2 (Th1) and IL-4 (Th2) responses [68, 69]. Perhaps more importantly, B7 prevents anergy [70]. It is, therefore, likely that co-stimulatory pathways are going to be of fundamental importance in designing tumour vaccines. Recently, the ubiquitous heat shock proteins have been shown to induce anti-tumour responses in a similar manner to HLA-transfected tumours. They may be presenting TADA peptides or providing a co-stimulus, or both [71–73].

## PRESENTATION

How are tumour vaccines to be presented? Morton's allogeneic cell lines are given following radiation intradermally under the axilla in multiple sites. Augmentation of the immune response with co-administered BCG, cyclophosphamide, cimetidine and indomethacin have all been tried [7]. The approach is eminently practical and without any significant toxicity. A number of ingenious delivery systems are now being tried to deliver cytokines, HLA and co-stimulatory cDNA *in vivo*, such as using cell-sized gelatin chrondrotin sulphide microspheres containing the cytokine, mixed with tumour cells, or giving the DNA directly into the tumour with liposomes [74–80].

## MELANOMA AND OTHER CANCERS

With several human trials reporting improved survival when antigens or cells are in the adjuvant or metastatic setting, lessons from the above animal experiments give grounds for hope that the status quo can be improved in the near future in melanoma. Trials giving IL-2 [81] and HLA B7 [75] transfected melanoma cells are already underway. Ideally, the goal for an effective cancer vaccine would omit the use of live or irradiated cells altogether and to this end, the search for melanoma-specific antigens to use as a vaccine has been pursued with vigour. Boon and colleagues cloned a tumour-specific antigen named MAGE-1 which is expressed on many melanomas as well as other types of tumours and no normal tissue with the possible exception of the testes. Interestingly, MAGE-1 is expressed on Morton's cell lines. The product of this gene has now been identified and two other MAGE genes identified (MAGE-2 and MAGE-3). More recently, Bakker and colleagues have cloned a glycoprotein gp100 from melanoma which is a specific target for melanoma-derived tumour-specific cell lines [15,82–84].

The interesting finding from the search for specific melanoma antigens is that like the previously established ganglioside and protein melanoma-associated antigens, the new ones are also present on other tumour cell types. Therefore, using melanoma-based vaccines against other solid tumours in an adjuvant setting where relapse is a compelling next step in such common tumours as colorectal, lung and breast cancer. In addition, they are likely to have their own specific antigens which may be targeted; for instance, carcinoma embryonic antigen, β-human chorionic gonadotrophin, α-fetoprotein, dopa decarboxylase, prostate-specific antigen, villin, *erb*-2, *erb*-3, CD30 etc., amongst many other possible targets.

Another way forward is to clone new tumour cell antigens from a variety of tumour types along the lines used by Boon and his colleagues [15]. VBeta-specific infiltration of a number of tumours should render this approach feasible [85–88]. Non-

MHC-restricted targets such as mucin may also make good candidates for vaccines as they are present in many epithelial tumours such as breast and pancreatic adenocarcinomas [89–91]. Moreover, mucin polymorphic mucins probably play an important role in the 'defence' of the tumour against the immune system.

## OTHER STRATEGIES

Many of the approaches being applied to inducing an effective T-cell response also have their counterparts in the humoral response. For example, the variable genes of the immunoglobulin molecules expressed on malignant B-cells have been rendered immunogenic by fusing them to GM-CSF [92]. A similar approach is under study for hepatoma in which hepatoma cells are fused with activated B-cells to create an effective anti-tumour response in the BERH-2 model in rats [93]. Weak B-cell epitopes have been made immunogenic by using an anti-idiotype approach such as against melanoma-associated proteoglycan (MPG) [94,95]. The ability to make recombinant hybrid molecules, such as immunoglobulin variable gene T-cell receptor agent, opens up endless possibilities for designing cancer vaccines. Inhibition of human colon cancer growth by antibody-directed LAK cells in SCID mice has recently been reported [96].

## FUTURE PROSPECTS

The ability to apply the fruits of the molecular revolution to the basic cancer vaccine approach has clearly given it a whole new lease of life. However, it is important to bear in mind that tumours are heterologous; even if they do express shared antigens, the tumours themselves may be limited by restricted MHC and lack co-stimulatory functions. Hence, population representative allogeneic cell lines should, in theory, help plug the potential "holes" in the immune response repertoire. Future prospects include the possibility of enhancing weak immune responses with immunisation of a cocktail of relevant peptides. This approach has been used to induce a CTL response specific for the p21 *ras* 61 mutation (and not unmutated ras) seen in many tumours [97]. A similar approach could be used for p53 mutations. Current tumour immunology dogma dictates that small tumour volumes are much easier to induce an immune-induced regression in, than bulky established tumours. Direct gene therapy approaches in such tumours may well be complementary. There are now several reports in which retroviruses containing the HSV tk gene are able to infect melanoma and brain tumours and induce tumour regression upon administration of ganciclovir which the tk gene converts to the toxic phosphorylated form [98–100]. However, what is most exciting is that the whole tumour can be killed even if only 50% of the cells are infected with the virus. This is known as the 'bystander effect'. Surprisingly, it is not all due to the imune response but appears to involve a cell–cell contact-mediated toxicity which may be due to cell-to-cell transfer of toxic molecules such as phosphorylated ganciclovir.

The next decade is going to see the resurgence of tumour immunotherapy. It will be made possible by the gene therapy techniques which allow the genetic manipulation *in vitro* of the vaccine cells [101,102]. This will allow a step-by-step approach to enable gene therapy approaches *per se* [103] (e.g. [98–100]) to be tried in the clinic. The possibility that combinations of immune-based treatments [104] may enable better control in a therapeutic situation will need to be borne in mind. Ongoing studies using known TADAs such as carcinoembryonic antigen (CEA) [105] may provide further practical targets, in addition to those being identified by molecular means. The application of molecular biology to the understanding of tumour immunology [106] may lead to the widespread use of cancer vaccine treatments in future, so that in 10 years time they could make up over half of all non-surgical treatments.


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