Review Article

The Development of Effective Tumour Vaccines

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Summary: *The search for effective tumour vaccines has been a very long one and has been fraught with many disappointments. The recent progress in our understanding of immune responses and tumour cell biology has produced new insights which many hope will lead to developing an effective cure for certain antigenic tumours. Tumour-associated antigens were first described many years ago. These antigens can be targeted by immunocompetent cells provided that an effective immune response, including antigen recognition, can be elicited. Therefore, tumour vaccines have been engineered such that there is better presentation of tumour-associated antigens and enhanced recognition. When coupled to effective adjuvants, anti-tumour responses were obtained in animal models and immune memory was elicited. These results and the ability to harvest professional antigen presenting cells from patients with different cancers have provided new hope for the development of anti-tumour stategies based on tumour vaccines. Clinical trials of tumour vaccines for prostate cancer and melanoma are reportedly in progress. The development of tumour vaccines from a hypothesis to near reality and the advantages of this form of treatment are discussed.*

Introduction

Effective tumour vaccines have been dubbed as the "Holy Grail" of immunologists and rightly so. This area of research has generated so much hope at times only to end up in failure and disappointment. Whereas vaccination for infectious agents is normally prophylactic, vaccination for tumours is normally therapeutic and is attempted only after diagnosis of the tumour. Thus, considering that tumours arising in different tissues have different biochemical and behavioural characteristics, tumour vaccines have to be tailor made for the different tumours in genetically distinct populations because different races may have genetically-linked discreet differences in immune functions. Tumour cells originate in normal tissues due to an accumulation of DNA damage which renders the cells independent of the growth control mechanisms which normally govern the life-span of healthy cells. DNA damage can be induced chemically, virally or through other agents such as exposure to UV radiation.

One of the many differences between normal and malignant cells is that tumour cells often express cell surface molecules or antigens which are usually expressed by developing tissues. The expression of these molecules is switched off in mature cells. This has led to researchers describing carcinogenesis as a process of de-differentiation. The surface molecules are expressed because the alterations in DNA function accompanying malignant cell development indiscriminately affect genes including those which code for cell surface antigens. It is also thought that de-differentiation reflects the lack of ability to undergo terminal maturation by malignant cells. The surface antigens predominantly expressed by tumour cells, have been labelled tumor-associated antigens but are not antigens specific to that tumour only. Normal tissues also express tumour-associated antigens (TAA) but do so in very small quantities or only transiently, for example during the embryonic stage. The possibility that TAA may be targeted by the immune system leading to immunologically-mediated tumour regression is the ultimate goal of this area of research.

The idea of applying tumour vaccines as an anti-cancer therapy was originally met with scepticism because it was firmly believed that the immune system would never react against self antigens (including TAAs) and therefore, the application of tumour vaccines was contradictory to this belief. Cytotoxic T lymphocytes (CTL) which are antigen-specific killer cells and are potentially active against self antigens, were thought to be eliminated or down-regulated before these could harm normal tissues Grooten et al, 1987). It was believed that CTL could not be generated against antigens which are shared between normal and malignant tissues (Dighiero and Rose, 1999). Animal models of the disease confirmed that CTL can be generated against self antigens provided there is a strong enough stimulus which overcomes tolerance to self antigens (Kurts et al, 1999). More recently, it was also shown that some cutaneous lesions in melanoma patients do contain CTL specific to melanoma antigens (Jager et al, 1999; thor Straten et al, 1999). Furthermore, some TAA may be viral products especially in tumours where a viral infection has been implicated as being the inducer of the malignancy or as being an accomplice in the process. These advances have added strength to the argument that tumour vaccines may have a deserved place in anti-cancer therapy, but as with other anti-cancer therapies, this will depend on the type of cancer and its characteristics.

Antigen recognition and the generation of anti-tumour immunity

Immune responses in humans can be generally classified into the humoral (or antibody) response and the cell-mediated cytotoxic response. In anti-cancer immune responses, the ultimate aim is to induce antigen-specific

CTL which will kill tumour cells but leave normal cells unharmed. This should also generate immune memory and theoretically prevent recurrence of the tumour. In viral infection, infected cells expressing viral antigen will die after producing viruses and the dead cell debris will be taken up by antigen-presenting cells (APC). Viral antigens will be processed into small 8-9mer amino acid sequences and presented to CD4+ T helper cells in association with major histocompatibility (MHC) class ?? molecules. The T helper cells will produce interleukin 2 (IL-2), the principal T cell growth factor, and expand the T cell population. Other cytokines such as interferon gamma (IFN-?) are produced by the T helper cells in what is called a T helper type 1 (Th 1) response and are thought to be required for the functional development of the cytolytic T cells. APC will also interact with CD8+ T killer cells through MHC class I molecules and educate these cells to kill target cells expressing the viral antigen. Antigen-specific CTL generated in this fashion will recognise infected cells and eliminate them before they shed viruses. This mechanism is also true for cell-mediated anti-tumour effects. APCs also take up tumour cell debris arising from processes such as apoptosis of tumour cells, and present them to CD4+ T helper cells. These helper cells will develop into Th 1 cells and with the help of further antigen presentation, induce a tumour-specific cell-mediated immune response.

Early attempts at vaccination of animal models

Clinicians noted that some tumours regressed spontaneously and even observed that some subcutaneous melanoma lesions regress while others at different sites in the same patient would progress. This indicated that the body might be mounting a protective anti-cancer response to the tumour and inducing its regression. These and similar observations led researchers such as Hiroshi Kobayashi in Japan to try and chemically, virally or radiologically modify tumour cells and produce a crude tumour vaccine for tumour-bearing animal models, a process Prof. Kobayashi labelled as xenogenisation of tumour cells. Prof. Kobayashi's group infected rat fibrosarcoma (KMT-17) cells with Friend leukemia virus and injected the cells into syngeneic (same strain) rats bearing solid tumours of the parental cells. The majority of the established tumours regressed and a significant number of rats were cured (Kobayashi et al, 1969). Eventually, through immunisation procedures, a tumour-associated antigen, CE7, was identified. This antigen was a self TAA which was not recognised by the rat immune system when expressed alone but was recognised when co-expressed with the Friend virus envelope protein on infected tumour cells. What was significant was that when the rats that rejected the tumour were again subcutaneously injected with the same parental KMT-17 tumour cells, the tumour failed to "take". This showed that the rats had developed specific immune memory and that the immune system could be recruited against self antigens if the immune stimulation (in this case the viral product) is strong enough to act as an adjuvant. Eventually, it was discovered that CE7 is shed from the KMT-17 tumour cell surface and may act as a decoy to immune responses (Chiba et al. 1989). Bleomycin and its analogues of anti-tumour antibiotics were found to inhibit CE7 shedding from the cell surface making the cells more antigenic in vivo (Micallef et al, 1992a). Besides this, Bleomycin is also immunostimulatory (Micallef et al, 1991; Micallef et al, 1992b) and tumour-bearing rats treated with Bleomycin reject tumour and develop immune memory (Micallef, 1993). These results indicated that chemical modulation of tumour cells in vivo by Bleomycin could also enhance tumour antigenicity and act as an adjuvant as well. Eventually, Shibata and co-workers showed that even irradiation from a 60Co source could inhibit CE7 antigen shedding and after fixation, this preparation proved to be an effective tumour vaccine in the KMT-17 rat fibrosarcoma model (Shibata et al, 1996).

The original observations of Prof. Kobayashi are very relevant to tumour immunology when one considers that there are strong implications for an association between certain viruses and tumour development. Certain papillomaviruses are associated with carcinoma of the cervix (Jarrett et al, 1990), human T-cell leukemia virus type 1 (HTLV-1) causes adult T-cell leukaemia (Poiesz et al, 1980; Yoshida et al, 1982), while Epstein Bar virus is associated with Burkitt's lymphoma (Hoffbrand and Pettit, 1984). Thus, modifications of tumour cell vaccines based on viral products may have potent anti-tumour effects in some cancers with a strong implication of viral involvement in the multistep process of carcinogenesis (Jarrett et al, 1990).

The function of the adjuvant

Some tumours may produce factors which inhibit the proper functioning of the immune system and result in local or systemic immunosuppression. Transforming growth factor beta (TGF-?) (de Visser and Kast, 1999), IL-10 (Howard and O'Garra, 1992; Chen et al, 1994) and prostaglandin E2 (Botti et al, 1998) are well known immunosuppressing factors produced by tumours or bystander cells influenced by the tumour cells. Furthermore, anti-cancer chemotherapy is non-specific and affects all replicating cells, including cells of the bone marrow which give rise to immunocompetent cells. Therefore, cancer chemotherapy is frequently accompanied by immunosuppression. Under such conditions, the immune system is too weak to act against a TAA in the absence of an immunostimulant. Even if the immune system is intact, a stimulus is still required to overrule the inhibitory signal which normally prevents the immune system from acting against self antigens, which include the TAAs. Thus it becomes obvious that an adjuvant is required to help the weakened immune system react against the antigen. The adjuvant should be potent enough to both overcome anergy of the T cells induced by tolerance to the tumour antigen, and induce a Th 1 immune response. Some researchers have administered Th 1 cytokines such as interleukin 2 combined with tumour-infiltrating lymphocytes (TIL) directly in an attempt to enhance cell-mediated immune responses (Rosenberg et al, 1988). The problems with this approach are the dose-limiting side effects induced and that the cytolysis appears to be non-specific in vitro (Schomburg et al, 1992).

Early clinical applications

Early clinical trials employed modified autologous (self) tumour cells such as Newcastle Disease Virus (NDV)-infected colon carcinoma cells but these did not produce

antigen-specific effects (Patel et al, 1992). In contrast, when patients with renal cell carcinoma were vaccinated with autologous tumour cells, the majority of patients who developed delayed-type hypersensitivity (DTH) towards the antigen also had prolonged survivals when compared to the patients who did not develop DTH (McCune et al, 1990).

Dr. Donald Morton at the John Wayne Cancer Institute in California was convinced that tumour vaccines could be applied against melanoma, an aggressive form of skin cancer. After screening a large number of melanoma cell lines, Dr. Morton selected 3 cell lines which were the most antigenic. This meant that these three cell lines between them expressed a spectrum of melanoma TAAs and could be used for vaccination. Bacillus Calmette Guerin (BCG), an attenuated form of the tubercule bacillus, was chosen as an adjuvant and Dr. Morton proceeded to immunise patients subcutaneously with a cocktail of the three antigenic cell lines and BCG (Morton et al, 1992). It must be stated that it is now evident that BCG can stimulate the production of cytokines which normally induce Th 1 responses and cell-mediated immunity, such as IL-12 (Wang et al, 1999) and IL-18 (Okamura et al, 1995). In fact, both IL-12 (Brunda et al, 1993) and IL-18 (Micallef and Kurimoto, 1999) have been used successfully as anti-cancer therapy in animal models. Remarkable responses and regression of metastases were observed in a number of patients treated according to Dr. Morton's protocol, however, this was not always effective and its efficacy appeared to be related to the characteristics of the different tumours, the tumour burden and the degree of metastasis present. These inconsistent results have hindered the general application of this form of treatment for melanoma.

Conjugates of TAA with BCG were used to successfully immunise rats against a syngeneic hepatoma but both the BCG and the TAA alone failed to induce any tumour resistance (Crum et al, 1977). Thus, simultaneous stimulation with adjuvant and TAA induces direct activation of, and recognition of antigen by the rat immune system.

Tumour-derived antigenic peptides

The vaccination of cancer patients with live tumour cells presents us with several problems of the ethical type. Therefore, other researchers prepared tumour cell extracts using different methods. Low molecular weight peptides were synthesized, and animals were stimulated with the peptides alone or combined with adjuvant (De Matos et al, 1998). Immune cells were also stimulated with the peptide extracts to determine whether the immune cells could be induced to kill tumour cells in vitro (Ikemoto et al, 1999). In both cases, results showed that even such tumour cell extracts could induce killer cell activity which was specific to the cells from which the extracts were derived. Nevertheless, there is some evidence to show that live modified tumour cells are a better tumour vaccine than dead cells or their extracts in vivo. To generate a specific anti-tumour response, professional APCs are required. Professional APCs express the MHC class ?? molecules required to present processed antigens to T cells. In addition, professional APCs also express other accessory molecules such as CD80 and CD86 which are required as a co-stimulus to induce a response by the T cells. The latter molecules bind their ligands CD28 and CTLA-4 which potentiate and inhibit the immune stimulation, respectively. The most potent professional APC is the dendritic cell (DC) which can be generated from peripheral blood macrophages (M?) or bone marrow cells in vitro, based on the cytokine stimulus. Attempts have been made to generate DCs and stimulate them with crude tumour cell peptide extracts (Zitvogel et al, 1996) or TAA peptides (Mayordomo et al, 1995) before re-introduction into tumour-bearing animals. The stimulated DCs were found to induce anti-tumour responses. In other instances, allogeneic tumour cell lysates induced specific responses in melanoma patients (Mitchell et al, 1993).

Certain lesions in patients with melanoma regress without any apparent cause and some were found to harbour anti-tumour CTLs. Using these CTLs, researchers have identified the structure of the variable region in T cell receptors which recognise the melanoma-associated antigen presented on the HLA molecules. This led to the deciphering of the amino acid sequences of antigenic melanoma peptides presented to the CTL on HLA class ? molecules. Thus, peptides such as the MAGEs (van der Bruggen et al, 1991), the BAGEs (Boel et al, 1995), Melanoma/MART-1 (Coulie et al, 1994; et al, 1994a), gp100 (Cox et al, 1994; Kawakami et al, 1994b) and others were identified. Different peptides are presented on different HLA class ? sub-set molecules as targets for CTLs. What this implies is that there can be no single universal vaccine for a particular type of tumour but rather different vaccines for the same tumour depending upon the race involved (eg. oriental versus occidental). This is because different human races express different HLA molecules which would dictate the tumour peptide which can be best presented by the particular HLA molecules expressed by that race.

Heat shock proteins (HSPs) are a group of proteins whose physiological function is to chaperone antigenic peptides in cells. When HSP-peptide complexes were derived from murine tumours and used to vaccinate against the same tumours, a significant anti-tumour effect was noted in models of colon carcinoma and UV-induced spindle cell carcinoma (Tamura et al, 1997). HSPs on their own failed to induce an anti-tumour response in the animals.

Gene therapy

The potential genetic modification of tumour cells has provided new opportunities to develop tumour vaccines. The successful enhancement of rat tumour cell antigenicity after viral infection described above led Prof. Kobayashi to attempt to duplicate the results he obtained using the intact virus by transfection of the target cells with the viral env gene (Sugiura et al, 1988). This result was among the first to show that direct genetic manipulation of tumour cells could modify their antigenicity and produce a tumour vaccine. Soon after Itaya and co-workers showed that mouse tumour cells could be made more antigenic by expressing allogeneic MHC class ? molecules (Itaya et al, 1989). More

recently, Komata et al. transfected human glioma cells with the cDNA for the accessory molecule CD80 and these could stimulate allogeneic CD8+ T cells in the presence of IL-12, the principle cytokine responsible for the development of Th 1 responses and cell-mediated immunity (Komata et al, 1997). Studies such as these confirm the importance of accessory molecules and the induction of a Th 1 response in the generation of cell-mediated tumour immunity. Dendritic cells have also been genetically modified to express murine tumour-associated viral or self antigens and used successfully in treating tumour-bearing mice (Tuting et al, 1997). DCs were transfected with cDNA coding for human papilloma virus (HPV) 16 antigens which are associated with tumour-associated viral antigen E7 or with that coding for the tumour suppressor gene p53. These modified cells were used to treat mice bearing a HPV 16-transformed murine sarcoma or mutant p53-expressing sarcomas. Mice receiving the treatment were significantly protected from tumour growth and a significant number of the treated mice remained tumour-free throughout the experiments. In vitro experiments showed that treated mouse splenocytes that rejected the tumour could kill the respective parental cancer cells.

Transduction of human DC with a TAA-encoding gene would theoretically enhance antigen presentation by the cell. Reeves and co-workers showed that DC transduced with the gene for the melanoma-associated antigen MART-1, could activate MART-1 specific human T cells in vitro (Reeves et al, 1996).

The role of antibodies

Although most of this discussion has centred around specific T lymphocyte responses to tumour cell antigens, which are thought to be the predominant responses against cancer cells, there is reason to believe that antibodies may also play a role in anti-tumour responses. Monoclonal antibodies against melanomas have been shown to have anti-tumour activity (Chapman et al, 1990) and there is strong correlation between specific antibody production and overall survival after vaccination with a melanoma cell vaccine (Jones et al, 1996). Melanoma patients immunised with a melanoma cell vaccine could also mount an antibody response against melanoma antigens (Hoon et al, 1995). Antibodies to melanoma antigens of the IgM class can also directly kill melanoma cells in the presence of complement.

Conclusions

The search for effective tumour vaccines has been a long one, however, the results to date, coupled with our increasing understanding of immune responses have added strength to the quest for these potentially life saving therapeutic regimens. Immunostimulatory cytokines such as IL-12 and IL-18 may finally fill in the void that has been created by the lack of suitable adjuvants, required to stimulate the immune system. Clinical trials are now underway combining genetically modified tumour cells with potent adjuvants which, one hopes, will form the basis for the successful treatment of various types of antigenic cancers.

Because of the broad nature of this subject, new results are being announced continuously and although every effort has been made to be as comprehensive as possible in this review, undoubtedly there are areas of interest which have not been discussed. Nevertheless, I sincerely hope that this article will give some information on the state of affairs in this field of research.

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