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Tumour Immune Therapy: The Birth of a New Array of Cancer Treatment Strategies

Farzin Farzaneh

Department of Molecular Medicine, The Rayne Institute, Guy's King's and St Thomas' School of Medicine, King's College, London-UK

ABSTRACT

The potential ability of the immune system to recognise tumour tissue and to be able to help with the treatment of cancer has been suspected for a very long time. However, it is only in the last few years that the full anti-tumour potential of the immune system is beginning to be appreciated. This is due to a rapid growth in the understanding of the cellular and molecular biology of both cancer and the immune system. Tumour immunology is now poised to offer a number of different strategies for prevention and the induction of immune mediated rejection of tumours.

These developments have been the subject of a number of comprehensive and extremely informative recent reviews. The present article aims to highlight particular aspects of the recent developments in tumour immunology and immune therapy that are likely to be of interest to readers of Tanaffos. In particular the case for immune therapy of cancer is examined and a number of different immune therapy strategies that are currently under active investigation are described. For a more thorough analysis of the subject the reader is referred to a number of particularly informative recent reviews, and the references therein, that have been listed at the end of this article. ***(Tanaffos 2002;1(2) :7-14)***

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CANCER AS A CONSEQUENCE OF INADEQUATE IMMUNOLOGICAL RESPONSES TO MICROBIAL INFECTIONS

The increased incidence of cancer in transplant patients demonstrates the importance of immune surveillance in reducing the incidence of malignant disease. This increase is particularly striking in cancers with a viral aetiology. Therefore, immune mediated eradication of the microbial causes of cancer is likely to play a very important role in decreasing the overall incidence of malignant disease. Some of the most important of the already identified microbial causes of cancer are listed in Table 1. These include both viral agents such as Epstein-Barr virus associated with lymphoma and nasopharyngeal carcinoma, as well as bacterial agents such as *Helicobacter pylori* contributing to the development of gastric ulcers that can proceed to gastric carcinoma. It is now believed that microbial agents contribute to the development of about 15% of all tumours. Therefore, effective vaccination against these agents will offer protection not only against diseases that are directly caused by these agents, but also reduce malignancies that are associated with the

Correspondence to: Farzaneh F
Tel.: +44(0) 20-7848-5901; fax: +44(0) 20-7733-3877
E-mail address: farzin.farzaneh @ kcl.ac.uk

chronic presence of these infectious agents. This is most clearly demonstrable in the reduced incidence of hepatocellular carcinoma in children who are protected against HBV infection at birth by the administration of gamma globulin, and/or HBV vaccination. This prophylactic role for vaccination can of course be extended to other agents as soon as effective vaccination strategies have been developed for them. Recent successes in vaccination against human papilloma viruses make consideration of the case for the prophylactic vaccination of teenagers against human papilloma viruses an urgent issue both for biomedical scientists and health economists. The issue in the case of HPV being that induction of immunity by vaccination should reduce the incidence of cervical carcinoma and anogenital tumours. Many such chronic infections result in the permanent presence and expression of specific viral genes in the host cells. These viral antigens provide particularly attractive targets for tumour immune therapy.

Table1. Example of human malignancies with a microbial aetiology

Microbial Agent	Malignancy
Epstein- Barr virus	Lymphoma/Nasopharyngeal carcinoma
HTLV-1	Human T-cell lymphoma
Human papilloma virus	Cervical carcinoma
Hepatitis B and C Viruses	Hepatocellular carcinoma
Helicobacter pylori	Gastric carcinoma

TUMOUR ASSOCIATED ANTIGENS AS POTENTIAL TARGETS FOR IMMUNE RECOGNITION

Although bacterial/viral antigens provide particularly obvious targets for immune therapy of cancer, these are not the only available targets. There are also a large number of endogenous gene products that are known to be tumour-associated-antigens (TAA) against which immunological responses can be elicited. These range from normal proteins that are over-expressed in specific tumours (*e.g.* c-erbB2/neu) to produce of the most common abnormality in all malignancies (*i.e.*chromosomal instabilities, resulting in the expression of novel proteins such as BCR/ABL or PML/RARα in myeloid leukaemia - Table 2).

Table 2. Tumour associated antigens as potential targets for immune therapy

Molecular cause	Product
Over expression of normal gene products	Growth hormone receptors (e.g. c-erbB2), minor histocompatibility antigens
Ectopic expression of normal genes	Embryonic gene products (e.g. CEA, AFP) and cancer-testis antigens (e.g. MAGE, BAGE, NY-ESO-1, etc.)
Genetic mutations	Activated oncogenes and mutated tumour suppressors (e.g. RAS, P53)
Abnormal protein glycosylation	Mucinous proteins such as MUC-1
Expression of viral genes	Viral gene products such as E6 and E7 of Human papilloma viruses
Chromosomal instabilities	Novel peptides at junction of chromosomal Translocation gene products (e.g. BCR/ABL, PML/RARα).

The amino acid sequence at the junction of the two separate protein domains in such chimeric fusion proteins provide entirely novel, and tumour specific, antigens which may be recognised by the immune system.

The long list of tumour-associated-antigens (TAA) in Table 2 demonstrates that there is no shortage of potential targets for immune mediated recognition and rejection of tumours. However, tumours are derived from self tissue. Thymic maturation, as well as other processes involved in the induction of immunological self-tolerance, makes our immune system generally speaking unable to react against self. However, it is clear that at least under specific circumstances the immune system can be provoked into mounting particularly devastating attacks against self tissue, thus causing diseases such as autoimmune

diabetes, rheumatoid arthritis, etc. The induction of such tissue- or tumour-specific auto-immunity is the objective of all anti-tumour vaccination strategies. However, for this to be an achievable objective, it is necessary to be sure that cancer is not in fact the product of general or specific immune dysfunction. In other words, if cancer was in fact the product of an immunological blind spot in the recognition of the tumour, or more specifically the tumour associated antigens, there would be little prospect of being able to induce the immune mediated rejection of cancer.

IS CANCER THE PRODUCT OF IMMUNOLOGICAL SUPPRESSION?

Until recently, a highly suspected possibility was that cancer was in fact the product of either general or specific immune dysfunctions inhibiting immune recognition or destruction of the tumour. No doubt immunological suppression, in man and in experimental animals, does result in an increased incidence of malignancy. However, there is now compelling evidence suggesting that neither broad nor specific immunological blind spots are the cause in the vast majority of cancers. The most important elements of this evidence can be summarised as follows:

Cancer patients do not present with broad-spectrum immunological suppression. There is usually little evidence of opportunistic infections, with the exception of specific leukaemia and very advanced stages in tumour progression.

There is clear evidence of a heightened immune status in cancer patients. These include the presence of high titre immunoglobulines to a large repertoire of tumour- associated-antigens. There is also good evidence for the increased presence of tumour reactive $CD4^+$ and $CD8^+$ T cells in the peripheral blood, lymph node and tumours in a number of malignancies. In addition, there is clear evidence of increased expression of stress proteins that are able to stimulate the activation of natural killer (NK) and other cytotoxic lymphocytes.

Quantification of activated T-cells that are able to recognise tumour associated antigens in the context of the patient's own MHC molecules has demonstrated the presence of such T-cells in higher numbers in cancer patients than in control healthy subjects. These studies, based on the use of ELISPOT and MHC/antigen tetramer staining, provide the most direct evidence for the lack of an immunological blind spot in cancer patients.

There is also clear evidence that the presence of T-cell mediated immunological responses in cancer patients correlate with better tumour prognosis. Similarly, in a number of tumours (*i.e.* colorectal and renal-cell carcinoma) the presence of $CD8^+$ T-cells within tumour nodules correlates with better prognosis.

It is therefore reassuring to see that cancer is not usually the product of general or specific immunological dysfunctions. For instance, malignant melanoma patients have an expanded number of specific T-cell clones with the appropriate T-cell receptors capable of recognising melanoma associated antigens in the context of the patient's MHC molecules. In fact such tumour specific T-cell clones are frequently present in substantially larger numbers in cancer patients than in the healthy population. However, there is evidence of sub-optimal T-cell activation in cancer patients, probably due to the weak immunogenicity of many of the identified cancer antigens and due to sub optimal priming of T-cells by non-professional antigen presenting cells, such as the tumour cells. In addition, because cancer represents a large number of persistent targets (a situation similar to chronic microbial infections) it does appear to induce functional exhaustion and even clonal deletion in

advanced stages of cancer progression. The fact that T-cell mediated responses against representatives of each of the different classes of tumour antigens outlined in Table 2, is detectable in a subset of cancer patients, provides confidence that better stimulation of these immunological responses and induction of immune mediated tumour rejection should be an achievable objective.

TUMOURS CAN ESCAPE IMMUNE SURVEILLANCE

Tumours employ a large array of different mechanisms to escape immune surveillance. These range from the loss of expression or mutation of antigens against which an immune attack has been mounted, expression of immune suppressive cytokines as well as factors required for cell mediated inhibition of cytotoxic T-cells (e.g. expression of FAS ligand for the induction of apoptosis in the FAS receptor positive tumour reactive T-cells). They also produce soluble antigens as decoys and induce clonal exhaustion or depletion by the continued presentation of a large target. In addition there is an array of immunological mechanisms normally involved in the shutting down of immune responses, for instance after dealing with an infection. In cancer, the inadequate stimulation of an initial response and the chronic presence of a large target, results in the dampening of the anti-tumour immune responses by these very same mechanisms. Examples of these include the induction of T-cell anergy and/or apoptosis due to the appearance of inhibitory co-stimulatory receptors (*e.g.* CTLA4) on the surface of the activated T-cells, and the action of regulatory $CD4^+/CD25^+$ T-cells.

STRATEGIES FOR THE STIMULATION OF IMMUNE MEDIATED TUMOUR REJECTION

Over a hundred years ago the New York surgeon William Coley reported the treatment of malignant tumours by repeated injection of heat-inactivated bacteria or bacterial extracts. In the intervening period there has been some evidence, and a great deal of hope, for the stimulation of immune mediated therapeutic responses against cancer. What Coley and other pioneers of immune therapy have shown is that induction of pro-inflammatory, immune stimulatory responses, particularly when in the vicinity of the tumour, can result in better immune mediated anti-tumour responses.

Table 3. Mechanisms involved in tumour escape from immune surveillance.

- Loss of antigen processing and/or presentation capacity (including loss of MHC expression).
- Loss of expression or mutation of tumour antigens.
- Expression of secreted and cell bound immune suppressive factors.
- Expression of decoys such as circulating tumour antigens (e.g. CEA, MUC-1, PSA, etc.).
- Self-limiting nature of immune stimulation (i.e. inhibitory feed back mechanisms such as expression of CTLA-4 on activated T-cells), action of regulatory T-cells, clonal exhaustion and/or clonal depletion due to the finite life span of T-cell clones.

The intratumoural injection of BCG vaccine, for the treatment of bladder carcinoma, is one such approach which has become an established treatment procedure with a similar degree of efficacy to chemotherapy. Other, more systemic immune stimulations with cytokines such as interleukine-2, gamma interferon, etc., have shown that even a broad spectrum, non-specific activation of the immune system can enhance immune mediated responses against the tumour. These studies have shown notable, but alas usually incomplete, clinical responses against the tumour. There is also evidence of a beneficial clinical outcome following the re-infusion of T-cells that are isolated from the peripheral circulation, draining lymph nodes or the tumour itself (tumour infiltrating lymphocytes) and

expanded *in vitro*, either non-specifically or in the presence of specific antigens expressed by the tumour. The success of these strategies in enhancing immunological responses against the tumour, occasionally associated with a therapeutic benefit, has encouraged the development of a large number of different strategies for the induction of immune mediated rejection of cancer. Generally speaking these are either based on the non-specific stimulation of the immune system, or aim to stimulate antigen specific responses. The non-specific stimuli include *ex-vivo* stimulation/expansion of T-cells and their re-infusion back into patients, or administration of immune stimulatory cytokines for the *in vivo* stimulation of T-cells and reversal of the tumour specific dormancy that is detected in some tumour responsive T-cells. Alternatively, a variety of vaccination strategies are used to induce/stimulate tumour specific immune responses (see table 4 for summary). These include attempts to make the tumour "more visible" to the immune system (*e.g.* intratumoural introduction of DNA vaccines encoding various cytokines, chemokines, or even allo-antigens). Other strategies rely on the use of autologous or allogeneic tumour cells, either with or without further modifications that aim to make them more immunogenic, as whole cell vaccines. Other promising strategies use dendritic cells (DC), as professional antigen presenting cells. In the latter studies the DC are engineered by a variety of different methodologies to express tumour antigens. These include the direct addition of one or more tumour antigen derived peptides for presentation by the MHC molecules on the surface of the DC, co-culture of the DC with tumour lysate or fragments to promote the uptake, processing and presentation of tumour antigens, loading of the DC with RNA molecules encoding specific or even whole tumour cell proteins, transfection or infection with expression vectors encoding tumour associated antigens either alone or in combination with additional immune regulatory factors, or even the fusion of DC to isolated tumour cells to generate hybrid cells. The latter DC/tumour cell hybrids express the large repertoire of factors that are normally expressed by the antigen presenting cells and are required for the efficient induction of T-cell stimulation, as well as a broad spectrum of tumour antigens that are expressed by the tumour cell partner. Given the ability of DC to both stimulate and to suppress T-cell mediated responses, it is particularly important for the induction of tumour rejection to ensure that the DC based vaccine does in fact possess an appropriate immune stimulatory phenotype. This remains an area in need of much better understanding if it is to yield its full potential.

PROSPECTS FOR SUCCESSFUL IMMUNOTHERAPY OF MALIGNANT DISEASE

The success of vaccination against microbial agents associated with specific tumours (Table.1) has already reduced the incidence of the corresponding tumours (*e.g.* the reduced incidence of hepatocellular carcinoma in HBV vaccinated children). A second clear example of success is provided by the BCG mediated induction of immunological rejection of bladder carcinoma with an efficacy comparable to that obtained by chemotherapy. There is also a rapidly expanding body of evidence demonstrating increasing levels of success by various vaccination strategies, amongst which those based on the use of dendritic cells are particularly prominent. However, in spite of these notable successes, immune therapy of cancer is still based substantially on experimental approaches, with very limited evidence of clinical success and outright cure. This is, at least in part, likely to be due to the fact that for ethical reasons, the vast majority of cancer immune therapy trials are in very advanced patients who have failed other

treatment strategies. These are therefore patients that usually have bulky disease. This means large numbers of tumour cells that have evolved a broad array of resistance mechanisms against the agents to which they have been previously exposed, possibly including immune surveillance. Not surprising therefore, these late stage tumours do appear to employ a variety of strategies to escape immune recognition and/or rejection. As the feasibility and safety of different immune therapy strategies become better established in Phase-I clinical trials, it becomes more logical and ethically acceptable to apply these

Table 4. Cancer immune therapy strategies

Strategy	Example	Advantages	Disadvantages
Non-specific immune stimulation			
Cytokines: systemic or Local administration or production (gene therapy mediated)	IL-2	Ease of production/administration, relative safety at low doses	Limited evidence of success
Bacterial vaccines	BCG	Ease of production. Safety	Evidence of success limited to bladder carcinoma, poor patient compliance as very uncomfortable
Antigen specific vaccination strategies			
Peptides	Mutant RAS MAGE, etc	Ease of production. Safety	HLA specific. Weak immunogenicity. Poor CTL induction. Escape mutants.
Proteins	CEA, MUC-1	Relative ease of production. Safety. Reduced/no HLA restriction.	Good for antibody production, but poor CTL stimulation.
DNA	Allo-MHC, co-stimulators, cytokines, factors, etc.	Ease of production. Safety. Can include multiple antigens ± immune regulators.	As for peptides and proteins it is dependent on identification of tumour associated antigens, limited evidence of efficacy
Recombinant viruses	Retrovirus, adenovirus etc.	Potential for high immuno-genicity. Large choice of vectors with good immuno-logical properties. Potential to express multiple antigens and immune regulators.	Safety concerns. Limited evidence of clinical efficacy.
Recombinant bacteria	Attenuated salmonella	Potential for high immuno-genicity. Large payload, hence multiple antigens/ immune regulators.	Inadequate knowledge of the basic mechanisms involved. Poor tumour penetration due to large size.
Ex-vivo antigen loaded dendritic cells (DC)	Allo-/autologus tumour/DC hybrids	Good immuno-genicity further enhanced by stimulatory factors. No antigen identification required.	Individualised, patient specific, cell processing. Poor standardisation.
Whole tumour cells	Alogeneic melanom cells	Ease of production. Some evidence of efficacy in melanoma.	Poor standardisation. Dependent on presence of shared tumour antigens.

treatments at earlier stages in disease progression, particularly in poor prognosis conditions in which a minimal residual disease status can be established by other strategies such as surgery. The reduced production of immune suppressive factors, and the reduced chance of generating escape mutants of various kind, combined with the reduced number of cancerous cells that need to be destroyed in order to achieve tumour eradication, should substantially enhance the chances of greater clinical success. Finally, it is important to emphasis that the success of immune therapy of cancer is not entirely dependent on the direct eradication of the tumour cells. The immune system may also be induced to contribute in a number of other ways to the effective eradication of the tumour. One of the best examples of this is the recent success in vaccination against endothelial cells that participate in the formation of the neovasculature on which the tumour is dependent for its supply of oxygen and nutrients. Recent studies be Niethammer, Reisfeld and colleagues at The Scripps Research Institute (TSRI) have shown that vaccination against vascular-endothelial growth factor receptor-2 (VEGF-R2), expressed by a replication deficient salmonella typhimurium bacteria, can allow oral vaccination against VEGF, resulting in a substantial reduction in the rate of growth of previously established tumours.

In conclusion, there is entirely justifiable optimism for the successful stimulation of the "search and destroy" power of the immune system to derive therapeutic benefits against cancer. The birth of tumour immunology with the pioneering work of William Coley over a hundred years ago has now come of age. The major discoveries of the past two decades in tumour biology and in immune regulation have substantially quickened the pace of progress in tumour immunology and immune therapy. We are now on the brink of significant new breakthroughs in both vaccination based prevention and immune therapy mediated treatment of cancer.

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