Clinical review

Science, medicine, and the future

Cellular immunotherapy for cancer

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During the past decade, our rapidly escalating understanding of immune surveillance and an appreciation of the mechanisms by which tumours escape its notice have led to promising new strategies against cancer. This paper reviews the concepts behind current research into cellular immunotherapy for cancer, presents data from clinical trials, and discusses the potential of this treatment as an adjunct to conventional modes of cancer treatment.

Predicted developments

- Clinical trials of tumour cell vaccines in patients with minimal residual disease at high risk of relapse
- Translation of cellular approaches into reproducible clinical benefit
- Ability to assay more precisely the clinical and immunological response to cellular treatment
- Definitions of the most potent combinations of effector cells and cytokine enhancers

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Methods

All three authors are involved in research into cellular immunotherapy and gene therapy. We searched PubMed and Medline databases using the terms "cancer vaccines," "dendritic cells," and "lymphocyte therapy."

The rationale for cellular immunotherapy of cancer

The importance of the interaction between the immune system and cancer cells was recognised in the 1890s when William Coley used streptococcal cultures to treat patients with advanced sarcoma. These attempts to activate general immunity led to clinical responses. More recently, antibodies and T cells that identify tumour antigens have been isolated from patients with cancer. It is clear that the immune system is capable of recognising tumour cells.

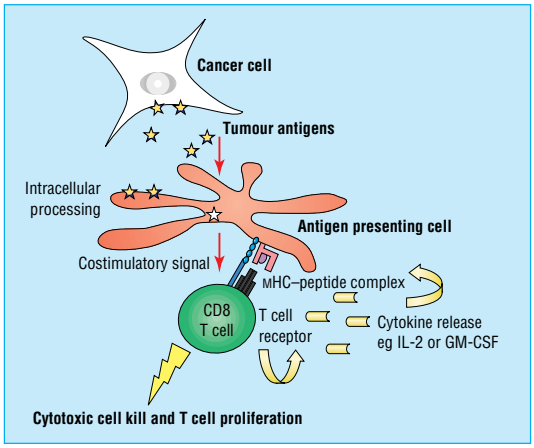

Fig 1 Antitumour immune response. Dendritic cells capture antigens released by cancer cells. After intracellular processing, antigenic peptides are loaded onto major histocompatibility complex (MHC) molecules on the surface of the dendritic cell. Specific T cells encounter these MHC–peptide complexes in conjunction with a costimulatory signal. The activated T cells proliferate and secrete cytokines, resulting in the production of a cascade of immune effector cells (IL-2=interleukin 2; GM-CSF=granulocyte-macrophage colony stimulating factor)

Cellular immunotherapy consists of giving the patient cells that stimulate antitumour activity in the patient (tumour and dendritic cell vaccines) or that have intrinsic antitumour activity (autologous and allogeneic lymphocytes). The aim is to harness potent immunological weapons to destroy cancer cells.

The immune response to cancer

Cytotoxic T lymphocytes are one of the critical effector cells that are able to lyse tumour cells. Receptors on the surface of T cells recognise antigens presented as peptide fragments on the surface of the class I major histocompatibility complex. Recognition of an antigen by a naive T cell bearing an appropriate T cell receptor is insufficient in itself to trigger activation of the T cell—the antigen must be encountered in conjunction with a costimulatory signal. In the absence of this, T cells become tolerant to the antigen.

Cellular orchestrators of T cell activation are professional antigen presenting cells (dendritic cells) that possess a remarkable ability to stimulate the immune response. These highly specialised cells capture and process antigens that are released during tumour cell breakdown and present them to antigen specific T cells. Once activated, the T cells, including CD4 T helper cells,

Reasons for the failure of immune responses against tumours

Impaired tumour recognition by immune cells

- Variable expression of tumour antigens
- Loss of expression of class I major histocompatibility complex, resulting in T cells failing to recognise tumours

Poor tumour immunogenicity

- Many tumour antigens are self antigens and as such are poorly recognised by T cells
- Lack of costimulatory molecules on tumour cells, which results in failure to stimulate T cells

Tumour "counterattack"

- Tumour cells secrete immunosuppressive cytokines (transforming growth factor β or interleukin 10, for example)
- Molecules expressed on the surface of tumour cells (for example, Fas ligand) may induce lymphocyte death
- Evolution of variants of tumour cells that do not express antigens

proliferate and secrete cytokines such as interleukin 2 and granulocyte-macrophage colony stimulating factor. These cytokines are potent stimulators of T cell proliferation and activation and give rise to a cascade of immune effector cells (fig 1).

Despite these highly developed responses, effective immunity against cancer frequently fails to develop—in effect, the immune system becomes blinded to the tumour. The ultimate aim of cellular immunotherapy is to overcome the failed immune response and get the immune system to effectively destroy the tumour cells.

Tumour antigens

As a target for cancer immunotherapy, the ideal tumour antigen is immunogenic and expressed exclusively on tumour cells. Tumour specific antigens include viral antigens and mutated gene products (table). Most known tumour antigens are expressed, to some degree, on normal tissues, and they are therefore "tumour associated" rather than truly tumour specific.

Potential sources of tumour antigens

Category of antigen	Example	Associated tumour
Expression of oncofetal antigens	Carcinoembryonic antigen	Colorectal cancer
	MAGE gene family products*	Melanoma and breast cancer
Tissue specific differentiation antigens	MART-1†	Melanoma
	Glycoprotein gp100	Melanoma
Oncogene/tumour suppressor gene products	p53	Many cancers
	HER-2/neu oncogene	Breast and ovarian cancer
	bcr/abl	Chronic myeloid leukaemia
Viral proteins	Human papilloma virus	Cervical cancer
	Epstein-Barr virus	Burkitt's lymphoma Hodgkin's disease
	Hepatitis B virus	Hepatocellular cancer

*MAGE=melanoma antigen.
†MART-1=melanoma antigen recognised by T cells; also known as Melan A.

Tumour cell vaccines

Whole tumour cell vaccines

Whole tumour cells, rendered safe by irradiation and mixed with an immunological adjuvant, were one of the earliest forms of cellular therapy. This approach avoids the need for tumour antigens to be identified before treatment and allows all of the relevant antigens to be included in the vaccine. Initial clinical studies showed the safety of this approach, with side effects mainly limited to erythematous reactions at the site of the vaccine.

Whole tumour vaccines are now entering phase III trials. One research group vaccinated patients with Dukes's type B and C colorectal cancer with autologous tumour cell vaccines mixed with BCG vaccine.[1] Although there was no difference in overall survival, significant improvements were seen in recurrence free survival in vaccinated patients, with the most benefit seen in patients with the lowest tumour burden. In patients with melanoma and renal cell carcinoma, the results from phase I and II trials have shown possible survival benefits when compared with those from historical controls.[2 3]

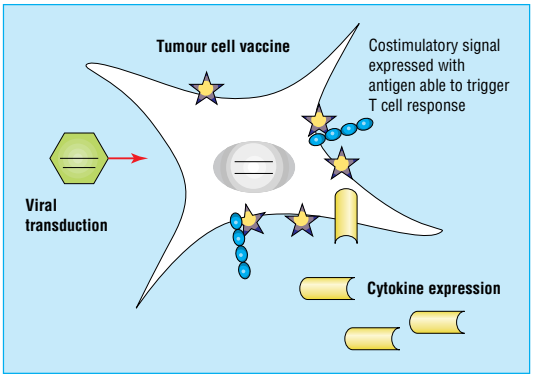

Fig 2 Tumour cell vaccines. Immunogenicity of tumour cell vaccines can be improved by transducing the tumour cell with genes that encode key components of the immune response (cytokines such as granulocyte-macrophage colony stimulating factor (GM-CSF) and costimulatory molecules)

Gene modified vaccines

A more recent approach is the use of vaccines containing genetically modified cells—gene modified vaccines—in which genes encoding key components of the immune response can be introduced into the tumour cells in vitro to increase the immunogenicity of the vaccine (fig 2). The most common gene modified vaccines use cytokines—the cytokine is produced in high concentrations in the vicinity of the tumour cells, where it alters the local immunological environment and enhances the activities of antigen presenting cells and the activation of tumour specific T cells. This approach avoids the side effects associated with systemic treatment with cytokines.

A phase I trial evaluated a tumour vaccine that secretes autologous granulocyte-macrophage colony stimulating factor in patients with metastatic renal cell cancer. This trial provided preliminary evidence of the benefits of this technique, with significant tumour regression in one patient and minimal side effects related to the vaccine.[4]

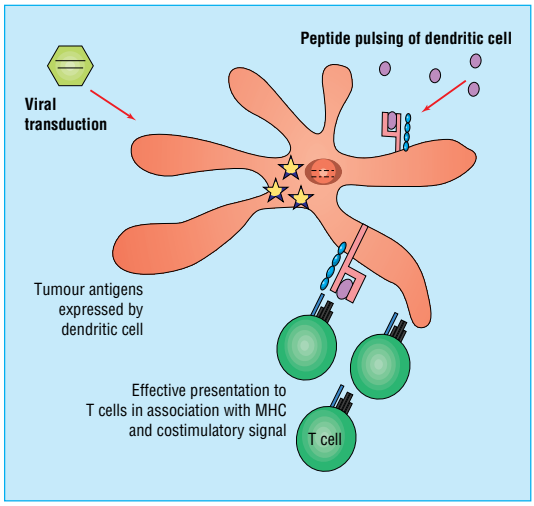

Fig 3 Dendritic cell vaccines. Dendritic cells generated ex vivo from a patient's peripheral blood monocytes or CD34 haemopoietic stem cells can be loaded with tumour antigens and reinfused into the patient with the aim of generating effective antitumour immunity. Loading of antigen can be achieved by a variety of methods, including pulsing cells with antigenic peptides or infecting the cells with recombinant viral vectors (MHC=major histocompatibility complex)

Generation of autologous tumour vaccines is expensive and labour intensive, and not all primary tumours can be expanded to produce enough cells for use in vaccine therapy. An alternative strategy uses gene transduced tumour cell lines as generic vaccines. In these, antigens common to the cell line and the patient's tumour act as targets for an immune response. Tumour antigens are presented to T cells by the host's (the patient's) antigen presenting cells in association with the host's major histocompatibility complex; compatibility of major histocompatibility complex between the patient and the vaccine is therefore unnecessary.[5]

Dendritic cell vaccines

Immunity produced by vaccines depends largely on the efficiency of the antigen presenting cell that initially processes and presents the antigen. Dendritic cells are probably the means by which most vaccines work; they possess an extraordinary capacity to capture and process antigen and contain all that is needed to stimulate T cell immunity, including high levels of major histocompatibility complex, costimulatory molecules, and adhesion molecules. These properties, coupled with the fact that it is now possible to generate, ex vivo, large numbers of functional dendritic cells from a patient's peripheral blood monocytes or CD34 haemopoietic stem cells, have led to considerable interest in the use of dendritic cell vaccines as a means to induce antitumour immunity.

Dendritic cells loaded with tumour antigens in the form of peptide fragments (fig 3), whole antigens, or tumour cell lysates are beginning to enter clinical trials, with some encouraging results. Patients with metastatic melanoma have been vaccinated with dendritic cells loaded with a cocktail of tumour specific peptides or tumour lysates, together with a chemical adjuvant to boost the immune response. In 16 patients, three had complete responses and two had partial responses.[6] Metastatic renal cell carcinoma has been a target for vaccination with a hybrid cell vaccine consisting of autologous tumour cells fused to dendritic cells. Despite the poor prognosis for such patients, objective clinical responses, including four complete remissions, were seen in 7 of 17 (41%) patients.[7] Ongoing clinical trials are using dendritic cells in renal cell carcinoma, prostate cancer, and melanoma.[8]

Gene therapy techniques can be applied to dendritic cell vaccines; such techniques use recombinant viral vectors that are incapable of replication to provide efficient and reliable means of gene transfer. Genetic material is introduced into dendritic cells to provide them with a renewable source of antigen for presentation; this should lead to more sustained expression of antigen. The expression of viral (and therefore foreign) genes may boost the immune response, but this antiviral immunity primed by dendritic cells may cause the immune system to destroy dendritic cells rapidly in subsequent rounds of immunisation. One solution may be to use viral vectors that do not result in the expression of viral genes, such as retroviruses or "gutless" adenoviral vectors.

Autologous T lymphocyte therapy

The use of interleukin 2 in the treatment of renal cell cancer and melanoma proved that an immunological treatment is capable, in some cases, of inducing long term regression of metastatic tumours. The mechanism by which these remissions occur is believed to be through the stimulatory effects of interleukin 2 on T lymphocytes.[9] Further research showed that tumour infiltrating lymphocytes, isolated from tumour samples

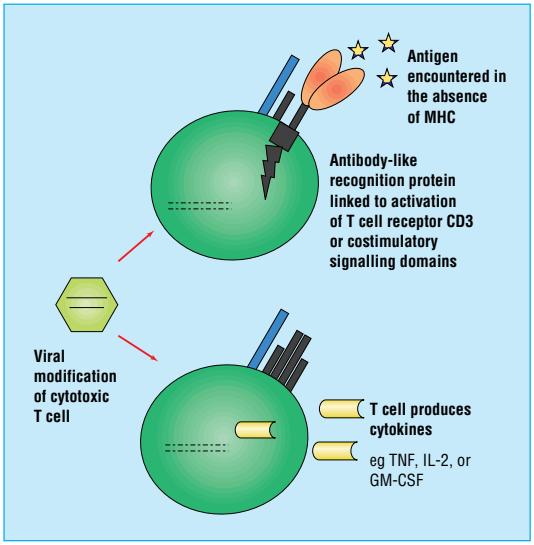

Fig 4 Gene modified T lymphocyte therapy. Lymphocytes can be transduced with genes that encode chimeric receptors consisting of extracellular antitumour antibody fragments linked to intracellular T cell receptors[11] or costimulatory signalling chains.[12] Contact with the tumour leads to proliferation and activation of the antigen specific T cells. Lymphocyte survival and antitumour efficacy may also be improved by the use of genes encoding various cytokines (MHC=major histocompatibility complex; TNF=tumour necrosis factor; IL-2=interleukin 2; GM-CSF=granulocyte-macrophage colony stimulating factor)

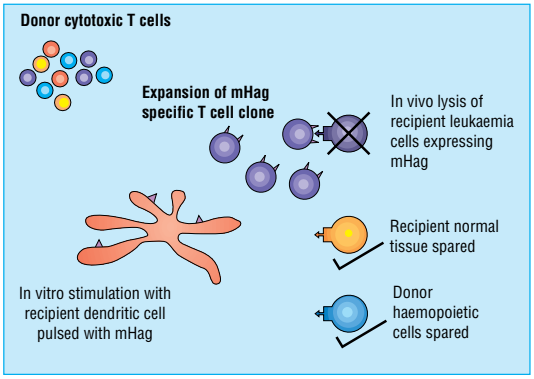

Fig 5 Adoptive immunotherapy with cytotoxic T lymphocytes specific for minor histocompatibility antigens restricted to the haemopoietic system. Responder lymphocytes from the allogeneic donor (who may be a sibling or unrelated matched donor) are stimulated by dendritic cells pulsed with antigens expressed specifically by haemopoietic cells from the recipient. This leads to the expansion of a specific T cell clone that is able to lyse recipient haemopoietic cells but is unable to attack the recipient tissues, which are susceptible to graft versus host disease. (mHag=minor histocompatibility antigens)

and grown in interleukin 2, could also induce remissions in these disease groups. Disappointingly, in patients receiving interleukin 2, the infusion of these cells did not improve response or survival rates significantly compared with those receiving interleukin 2 alone.[10]

More recently, advances in the ex vivo use of gene transfer technology to genetically modify lymphocytes have made it possible to increase their effectiveness. One strategy involves fusing the antigen recognition domains of specific antitumour antibodies with intracellular T cell receptor signalling chains to form "chimeric" T cell receptors (fig 4). Cytotoxic T lymphocytes modified to express such receptors are specifically activated on contact with tumour antigen, without the need for tumour expression of major histocompatibility complex. T cells genetically modified in this way have been used successfully to treat human ovarian cancer cells in immunodeficient mice,[11] and clinical trials are ongoing.

Other approaches being studied include increasing antitumour efficacy by modifying lymphocytes to secrete antitumour cytokines, such as tumour necrosis factor, and improving in vivo T cell survival through the autocrine production of growth factors such as interleukins.

Allogeneic lymphocyte therapy

A potent graft versus leukaemia effect may be mediated by donor T cells that recognise disparities between donor's and host's tissue histocompatibility antigens as well as tumour antigens. Infusions of allogeneic donor leucocytes led to clinical responses in 60-80% of patients with chronic myeloid leukaemia who had relapsed after allogeneic transplantation. Recent reports suggest that a graft versus tumour response may be successfully induced against solid tumours such as renal cell carcinoma.[13]

Unfortunately, use of allogeneic lymphocytes is frequently accompanied by graft versus host disease, in which donor T cells recognise the host tissue as "foreign." Novel approaches are being used to separate the graft versus leukaemia effect from the graft versus host effect, which should make giving donor leucocytes safer. Donor lymphocytes can be genetically modified to express genes that sensitise cells to specific drugs that can be administered to trigger cell death. This may confer the ability to eliminate effector T cells in the instance of toxic graft versus host disease.[14]

Specifically selected allogeneic donor cytotoxic T lymphocytes offer the prospect of an antileukaemia effect in the absence of graft versus host disease. One exciting approach may be the expansion ex vivo of those allogeneic cytotoxic T lymphocytes that are able to selectively recognise those minor histocompatibility antigens whose expression is restricted to recipient haemopoietic (and therefore leukaemic) cells (fig 5).[15] The Wilms's tumour gene WT1 is expressed at increased levels on the blast cells of patients with acute myeloid leukaemia and chronic myelogenous leukaemia. Current approaches are looking at the potential for exploiting WT1 as a target molecule, in order to selectively direct cytotoxic T lymphocytes against leukaemic blast cells.[16]

Limitations of cellular therapy

One concern with cellular immunotherapy is the induction of autoimmunity—vitiligo developed in 20% of melanoma patients who responded to interleukin 2.[17] Other evidence of autoimmune disease has not been seen in any of the cancer vaccine trials to date, but is a possibility. Inducing autoimmunity against organs for which replacement therapy is available, such as the pancreas, may be acceptable to patients who otherwise face the possibility of dying from their disease, but a more widespread autoimmune reaction could limit the use of some cancer vaccines.

We have discussed small pilot studies performed in specialist units, but it is important to prove clinical benefit in large, randomised studies. Cellular therapy is expensive, time consuming, and complex, and adopting this approach on a large scale will be challenging.

The future

Most clinical trials to date have vaccinated patients with advanced disease. These patients will have some degree of immunosuppression, from the cancer itself and as a result of previous treatment. Immunisation strategies are likely to be most beneficial when applied to patients with minimal levels of disease and tumour types known to be particularly immunogenic, such as melanoma and renal cell carcinoma. Safety issues must be evaluated in patients where no conventional treatment is proved to be successful; however, as we move from the realm of pilot studies, it will be critical to design future trials to tackle the subject of residual disease burden, which may occur after surgery. Preliminary research suggests that these therapies will be less toxic than more conventional modes of treatment.

Cellular therapy is a rapidly evolving field, with incremental technological advances in cellular manipulation and genetic modification. As we attain a deeper understanding of the power of the immune response, we may be able to exploit this system and use it as a platform on which to build a successful therapeutic strategy to fight cancer.

Additional educational resources

Websites

CancerNet (www.cancernet.nci.nih.gov)

Information about cancer vaccine trials that are currently recruiting

Gene therapy advisory committee (www.doh.gov.uk/genetics/gtac)

UK gene therapy trials approved by the gene therapy advisory committee

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Miguel de Cervantes, hydropsy, and Thomas Sydenham

One day during the spring of 1616, as three friends quickly rode their horses from Esquivias to Madrid, a student hurried behind on a donkey and begged them to slow down. As they did so one of the gentlemen told the student that "Señor Miguel de Cervantes' fast mount" was the reason for their swift pace. The student, recognising Cervantes' paralysed hand, addressed him with admiration and devotion. The four resumed their journey and Cervantes wrote, "We talked about my disease," and quoted the student saying, "Your malady is hydropsy, something which cannot heal even if you were to drink as much sweet water as there is salt water in the entire ocean … Señor Cervantes, limit your drinking, without forgetting to eat—this will help you without having to take any remedy." Cervantes confirmed, "Many others have said so, but I can't avoid trying to quench my thirst, as though drinking were the very reason for which I was born," and he added, "My life is coming to an end, to go by my pulses—they'll stop beating on Sunday and I'll die."

Miguel de Cervantes Saavedra died four days after writing the above conversation in the prologue to his posthumous book *Los trabajos de Persiles y Sigismunda* (1617). He died in Madrid at the age of 68 on Saturday 23 April 1616 (on the same day as William Shakespeare). What caused his death? Does his biography give any clue to his last illness?

In 1569, aged 22, Cervantes contracted malaria near Rome and malaria still troubled him at Lepanto on 7 September 1571, when, despite high fever and his fellow sailors' advice to rest, Cervantes took part in the battle and was wounded. Two gunshots hit his chest, one crippled his left hand, hence his nickname "*el manco* (one handed) *sano* (unamputated)." His wounds had not yet healed when he was captured on 26 September 1575 off Aigues Mortes by pirates while sailing from Naples to Spain. He was kept prisoner in Algeria for five years, where, after each attempt at escaping, he was flogged and was once kept chained "from head to feet" for five months.[1]

If his medical history cannot explain Cervantes' hydropsy, can his writings do so?

Cervantes treats medical questions in his *Don Quijote de la Mancha* with such exactness that some have wondered whether he was a physician.[1] In his works he names over 100 medicinal plants and his description of Don Quixote's psychological development earned him the nickname "Raphael of medicine." Cervantes uses the term hydropsical—not hydropsy—once in his *Don Quijote*, meaning ascitic.

But did hydropsy mean anything else in Cervantes' time? The *Tesoro de la Lengua Castellana o Española*, published in Madrid in 1611, defines hydropsy as "a disease due to watery humour that swells the body" and adds that it also means avarice because "patients with hydropsy can drink as much as they like, they'll never quench their thirst, like misers, no matter how much they gain, cannot appease their greed."

Did Cervantes have polydypsia? Did he have diabetes, ascites, uraemia, anasarca, or heart failure? Heart disease seemed "logical" a century ago "since he had such a good [literally large] heart."[2]

The enigma remains. But its solution does not matter. More important is Thomas Sydenham's (the English Hippocrates') answer to his disciple Richard Blackmore's question what to read to become a better doctor: "read *Don Quixote*."

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