Tumours: Immunotherapy

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For many years, the treatment of cancer was primarily focused on surgery, chemotherapy and radiation, but as researchers learn more about how the body fights cancer on its own, antitumour immunotherapies are being developed. Although some therapeutic approaches in use today are nonspecific, most protocols are designed to be antigen specific; the latter can be accomplished by either adoptive transfer or vaccination. Recent preclinical and clinical studies reflect the effectiveness of immunotherapy in combination with chemotherapy as a potential approach to specifically target cancer leaving normal cells safe.

Introduction

The concept of modulating the immune system to achieve an antitumour response is not new, with numerous attempts documented throughout history. The first successful efforts at achieving an anticancer response via immunotherapy, however, were not achieved until the turn of the nineteenth to twentieth century when a surgeon named William Coley noted the regression of an unresectable sarcoma subsequent to a postoperative wound infection. Building on this observation, he was able to show the objective regression of a variety of tumours using bacterial (Streptococcus) extracts (Coley's toxins), presumably through a mechanism of nonspecific immune stimulation. Despite Coley's toxins treatment resulted in the encouraging results of shrinking sarcoma, it came under a great deal of criticism because many doctors did not believe his results. Even though this criticism caused Coley's toxins to gradually disappear from use, the modern science of cancer immunology revealed that Coley's principles were correct and that some cancers are sensitive to an enhanced immune system (McCarthy, 2006). As our understanding of the immune system and tumour immunology has expanded, we have developed the ability to apply specific immunotherapies designed to enhance the immune response against unique targets. These immunotherapies can be broadly divided into two categories: (1) adoptive cell transfer (passive immunotherapy), and (2) vaccination (active immunotherapy) (**Table 1**). Adoptive transfer involves the direct transfer of the actual components of the immune system already capable of producing a specific immune response. These components could be in a form of cell-based or antibody-based therapies. In the case of cell-based adoptive therapy, preconditioning the patients with chemotherapeutic drugs or radiation is used to enhance the efficacy of this treatment regimen. Vaccination is defined as the administration of a particular antigenic element to induce a specific immune response. Antigenic elements could be in different forms, including killed tumour cells, whole tumour cell lysate, antigenic tumour protein, defined antigenic peptide of the tumour protein antigen, or naked deoxyribonucleic acid (DNA) encoding the antigen of interest. In order to be effective, these components are often delivered to patients by different vectors, including retrovirus, adenovirus or dendritic cells (DCs). Recent preclinical and clinical studies showed that cancer immunotherapy could be more effective when adoptive cell therapy is followed by vaccination.

Nonspecific Immunotherapy

Immune responses are mediated by the act of both innate immune cells (nonspecific component) and adaptive immune cells (specific component) of the immune system. Therefore, nonspecific immunotherapy describes therapies, often microbial products that can result in activation of the nonspecific arm of the immune system without regard to any known tumour antigen. Targeting nonspecific therapy is crucial for the initiation of full functional specific immune responses. **See also**: Immune System: Manipulation *In Vivo*; Tumour Immunology

Danger signals

Cells of the immune system respond to 'danger' signals, which are often provided by the invaded microbes. These signals are perquisites for initiation of effective adaptive

Table 1 Different nonspecific and specific approaches of tumour immunotherapy

Approach	Description
Nonspecific immunotherapy	
• Toll-like receptor (TLRs)	Triggering of TLRs expressed on cells of innate immunity by their cognate TLRLs. This pathway stimulates innate immune cells and bridge innate and adaptive immunity
• Cytokines	Administration of cytokines that can lead to the destruction of tumours by a direct antitumour effect and/or an indirect modulation of the antitumour immune responses
• NK cell therapy	Infusion of alloreactive NK cells with haematopoietic stem cell transplantation, or infusion of *in vitro* IL-2 treated NK (lymphokine-activated killer; LAK) cells and IL-2
Specific immunotherapy	
I Adoptive transfer	
• Antibody adoptive transfer	Infusion of already made tumour-specific antibody that can kill tumour directly or induce complement activation and/or antibody-dependent cellular cytotoxicity
• Adoptive cell transfer	Infusion of tumour-infiltrating lymphocytes, obtained by culturing single-cell suspensions of T cells harvested from tumour tissues, concomitantly with IL-2
II Vaccination	
• Tumour cell-based vaccines	Vaccination with lethally irradiated tumour cells mixed with a potent adjuvant. Cells can also be transduced with cytokine or costimulatory genes that enhance their immunogenicity
• Tumour antigen-based vaccines	Delivery of crude or recombinant tumour antigen
• Peptide-based vaccines	Delivery of specific epitopes with a defined sequence that can bind to MHC class-I and class-II and recognized directly by CD8 and CD4 T cells, respectively
• Dendritic cell-based vaccines	Delivery of antigen or peptide-pulsed dendritic cells generated from bone marrow or peripheral blood. Cells are usually activated with maturation stimuli before injection
• Gene delivery-based vaccines	Delivery of naked DNA or viral vector DNA encoding tumour antigen
• Idiotype-based vaccines	Delivery of an antibody that mimics a natural tumour antigen The body can then respond to the idiotypic antibody with antigen-specific humoral response

immunity through linking the components of innate and adaptive immunity. Danger model was hypothesized in 1994 by Polly Matzinger suggesting that specific immune response develops as a result of danger detection rather than discrimination between self and nonself antigens (Matzinger, 1994). Recent studies, in particular in tumour setting presented ample evidence supporting this theory. Typical danger signals are microbial products (exogenous danger signals) that release upon microbial infection. Being under stress, cells themselves also release endogenous danger signals, including heat-shock proteins (HSPs), nucleotide, oxygen radicals, uric acid and inflammatory cytokines. In most cases, the immune system can mount vigorous immune responses against microbes, but not against cancer. Therefore, the challenge in cancer immunotherapy is how to manipulate the body's own immune system to fight cancer. In this context, successful immunotherapy and tumour rejection has been reduced in many cases to the creation of appropriate inflammation by danger signals. The majority of these danger signals are now recognized for their ability to serve as a secondary therapy or adjuvant to specific tumour immunotherapies. Indeed, recent studies provided wealthy information affirming that nonspecific components of the innate immune system are crucial for effective adaptive specific immunotherapies.

Bacille Calmette–Guérin (BCG)

The most widely used adjuvant in immunotherapy is the attenuated mycobacterial strain BCG. Initially used as a live and potentially infectious reagent, it provided an effective vaccine against tuberculosis. In the early 1970s it was found to have anticancer effects. After extensive clinical testing, it is recognized as having modest utility in only a few cancers, including metastatic melanoma and certain types of early bladder cancer. In an open-label clinical trial, when BCG was coadministered with a polyvalent tumour cell vaccine to patients with advanced colorectal

carcinoma, it resulted in a significant tumour antigen-specific IgM response. In a randomized clinical trial evaluating post-operative immunization with irradiated tumour cells in patients with colon cancer, a 44% reduction in tumour recurrences observed after 5 years of follow-up when BCG was utilized as adjuvant (Vermorken *et al*., 1999). BCG-cell wall has also been used in superficial bladder cancer and has been considered as the treatment of choice. BCG has also been fractionated into safer, individual, subunit components, such as the cell wall skeleton and trehalose dimycolate, both of which have been shown to potentiate an antitumour effect. Although effective (as whole or fractionated) in a limited manner as a primary therapeutic reagent, the greatest utility of BCG and its subcomponents probably resides in its ability to function as an adjuvant (or supplement) to other forms of therapy. Although the exact mechanism responsible for generating an anticancer response has yet to be determined, activation of macrophages, dendritic cells and lymphocytes occurs in response to BCG treatment. Presumably, this nonspecific immune activation stimulates the generation of a specific antitumour response. Recent studies explored that components of BCG, including cell wall skeleton and peptidoglycan, activate toll-like receptors TLR2 and TLR4 signalling leading to activation of innate immune cells. This supports the concept that triggering danger signals, in particular TLRLs, can effectively support cancer immunotherapy. **See also**: Vaccines: Whole Organism

Toll-like receptor (TLR) signalling

A fundamental difference between tumour and microbes is that only the latter encode products (signatures) that are recognized as danger signals by pathogen recognition receptors (PPRs) expressed in the innate immune cells. A typical example of the PPRs is a group of TLRs, so far about 13, that sense different classes of microbial ligands (TLRLs), including lipopolysaccharides (or its nontoxic derivative monophosphoryl lipid A, MLP) (TLR4L), Ribi.529 or β-defensin 2 (TLR2/6L), bacterial lipopeptides and the yeast cell wall zymosan (TLR1/2L), unmethylated bacterial and viral DNA (TLR9L), viral single- and double-stranded ribonucleic acid (RNA) (TLR7/8L and TLR3L, respectively), and flagellin (TLR5L) (Seya *et al*., 2006). Triggering of TLRs by their specific TLRLs alarms the innate immune cells (namely DCs, macrophages and natural killer (NK) cells), which in turn activate T-cell responses. The current concept now is to alarm immune cells or to modify tumour cells in a way that the immune system will recognize them as dangerous and destroy them. Mimicking the antimicrobial immunity, recent preclinical and clinical studies have established that provision of different forms of danger signals, in particular TLRLs, systemically or into tumour environment itself, profoundly awaken the cross talk between innate and adaptive immunity, and thus activated T cells to recognize tumour as a danger. TLRLs have been used successfully as immunomodulators with or without tumour antigen administration. Engagement of TLRs such as TLR7/8 and TLR9 have shown potential adjuvant antitumour therapies in clinical settings. For instance, the addition of CpG DNA (typical TLR9L) to a melanoma vaccine resulted in an effective cytolytic response (Speiser *et al*., 2005). Imiquimod, a synthetic TLR7/8L, has been successfully used in the treatment of basal cell carcinoma. In an open-label trial, topical imiquimod was found to enhance the immunogenicity of vaccine containing Flt3 ligand and a melanoma peptide (Shackleton *et al*., 2004). MPL, a TLR4L, has been used as an adjuvant in clinical trials of vaccines against melanoma, glioma and pancreatic and colorectal carcinoma, inducing substantial tumour-specific immunity in response to vaccination (Shackleton *et al*., 2004). These recent studies provide examples illustrating the potent adjuvant effects of triggering of TLR signalling pathways on responses to cancer immunotherapy. Furthermore, requirement of danger signal, represented for example by TLR signalling, for effective cancer immunotherapies would explain why Coley's toxins and BCG are effective adjuvant therapies, since both encode TLRLs. Moreover, a recent preclinical study clearly showed that yellow fever vaccine YF-17D, one of the most effective vaccines available with a 65-year history of use in >400 million people globally, activates multiple DCs subsets via TLR2, 7, 8 and 9 to stimulate polyvalent immunity (Querec *et al*., 2006). Eventually, further studies are required to explore whether TLR–TLRL interaction is efficacious towards different tumour types.

Cytokines

The recent cloning of cytokine genes has allowed for their large-scale production and administration to patients with cancer. Cytokine therapy can lead to the destruction of tumours by one of two general mechanisms: (1) a direct antitumour effect or (2) an indirect modulation of the antitumour immune responses. In the first, cytokines directly interact with tumour cells leading to either apoptosis, inhibition of cell division, or blocking tumour angiogenesis (formation of new blood supplies to the tumour site). Typical cytokines such as tumour necrosis factor alpha (TNFα), interferon alpha (IFNα), IFNβ, interleukin 4 (IL-4), IL-6 and IL-12 have all been implicated in this mechanism. Although effective as singular agents, the combination of multiple cytokines can be even more beneficial by acting against tumour cells in an additive or synergistic fashion. Some cytokines, such as TNFα and IL-6, are able to suppress the growth of some tumours while actually promoting the growth of others. As such, the administration of cytokines demands great care. Cytokines utilizing the indirect mechanism mediate

tumour regression by stimulating or activating immune cells, which can then mediate an antitumour response through a variety of pathways. Some cytokines can enhance or activate particular types of immune cells, such as IL-2, which promotes T-cell and NK cell growth. Other cytokines such as the interferons and granulocyte–macrophage colony-stimulatory factor (GM-CSF) can act on professional antigen-presenting cells (APCs) and upregulate markers such as major histocompatibility complex (MHC) molecules and the costimulatory molecules CD80/CD86 (B7 family) and CD40 that have important roles in facilitating the activation of lymphocytes. These examples are not an exhaustive list of cytokines or their functions, as there is much still to be learned. Recent studies provided solid evidence for the efficacy of new cytokines, including IL-15, IL-21 and IL-23, and different chemokines and growth factors, to enhance antitumour immunity. There are a large number of cytokines being tested in humans for anticancer therapy, including IL-1, IL-4, IL-6, IL-7, IL-11, IL-12, macrophage inflammatory protein (MIP)-1α, IFNβ and IFNγ. IL-2 is approved by the US Food and Drug Administration (FDA) for the treatment of metastatic disease in both of these histopathologies. In patients with metastatic melanoma or renal cell carcinoma, intravenous IL-2 can induce objective tumour regression in 17% and 20% of cases, respectively (Rosenberg *et al*., 1994). Furthermore, clinical studies have demonstrated that combining IL-2 with other cytokines, such as IFNα, may lead to an enhanced response. IFNα is FDA approved for the treatment of malignant melanoma, chronic myelogenous leukaemia, hairy cell leukaemia and Kaposi sarcoma. Another cytokine, TNFα, although toxic systemically at therapeutic doses, can be effective when administered regionally via isolated limb perfusion to treat extremity melanomas and sarcomas. Growth factors, in particular GM-CSF, G-CSF, Flt3 ligand and IL-7 which are FDA approved play an important role in supportive therapy following bone marrow transplantation by facilitating quicker reconstitution of the immune system and improving patient survival. In addition, GM-CSF and Flt3 ligand indirectly support active immunotherapy in cancer patients through mobilization of sufficient numbers of DCs, which are crucial for generation of tumour-specific immune responses upon vaccination (Vuckovic *et al*., 2002). Several chemokines, in particular secondary lymphoid chemokine (SLC, CCL21), have been utilized to target T cells and DC trafficking to lymph nodes, a perquisite site for these cells to meet and to mount immune responses upon vaccination. Cytokines, in particular IL-7 and IL-15, have been found to significantly enhance the survival and turnover of the tumour-specific T cell memory responses, which is crucial for the longevity of efficacious antitumour immunity. Of note, recent studies revealed that provision of cytokines is necessary for efficacious combinatorial treatments with different cancer-based immunotherapy, including preconditioning the cancer patients with chemotherapeutic drug followed by adoptive transfer or active vaccination. Indeed, accumulating data from preclinical studies provided evidence suggesting that cytokines, chemokines and growth factors contribute to all phases of the antitumour immune responses, including initiation, generation, differentiation and establishment of preventive immunity. More clinical trials, however, are necessary to determine dose-limiting toxicities and to predict immunological responses in the more complex *in vivo* environment.

Cytokines are often delivered by systemic injection, which associates with significant toxicity. Compared to systemic administration, paracrine release, defined as targeted local delivery, of these cytokines has the potential to enhance efficacy while decreasing the likelihood of associated toxicities. One means of achieving paracrine cytokine delivery is to introduce a single or multiple cytokine genes into a host cell such as a fibroblast, DCs or tumour cell. Paracrine cytokine delivery approach has been able to significantly increase the efficacy of several tumour cell-based vaccines, and several cytokine gene-modified autologous tumour cell-based vaccines are being evaluated in phase I clinical trials. Most of these paracrine delivery schemas, however, are based on viral vector-based delivery, which although effective, it has limitations including the generation of neutralizing antibodies in addition to lacking the simplicity and versatility required for universal clinical application. Seeking a simple, effective and inexpensive methodology, several nonviral approaches have been studied for cytokine delivery, including naked DNA, liposomes, polymers, lipids, silicone and alum-based delivery (Salem *et al*., 2006). Both preclinical and clinical studies have shown that controlled-release by different injectable polymers in a soluble or gel form can provide a clinically feasible alternative to gene modification for local, sustained delivery of several cytokines, including IL-1, IL-2, GM-CSF, IFNα and TNFα. The cytokines, in protein or DNA forms, delivered by polymers are functionally active with stable adjuvant effects to components of immune systems. Some of these approaches have been in clinical trials and the results hold potential application in improving the beneficial effects of cytokine-based immunotherapy. **See also**: Antigen-presenting Cells; Apoptosis: Molecular Mechanisms; Cytokines; Haematopoietic Growth Factors; Haematopoietic Growth Factors: Therapeutic Uses; Interferons: Therapeutic Uses; Interleukins; Melanoma; Tumour Necrosis Factors

Cell therapy

Nonspecific immunotherapy can also be achieved via live cell transfer. In patients with metastatic melanoma, human peripheral blood mononuclear cells can be isolated and cultured with IL-2 to generate a class of cells denoted as lymphokine-activated killer (LAK) cells. These cells phenotypically and functionally resemble NK cells. When

LAK cells are administered concomitantly with IL-2 into patients with either advanced metastatic melanoma or renal cell carcinoma, complete tumour regression can be achieved in about 10% of cases (Rosenberg, 1986). The reduced levels of MHC expression often found on tumours may make them especially susceptible to lysis by NK cells.

Beside adoptive LAK cell therapy, recent preclinical and clinical studies have also established the beneficial effects of adoptive NK cell therapy to treat leukaemia (Velardi *et al*., 2004). In haematopoietic stem cell (HSC) transplantation for acute myeloid leukaemia (AML), donor and recipient pairs are identical for one human leukocyte antigen (HLA) haplotype and incompatible at the HLA class I and II loci of the unshared haplotype. Therefore, upon HSC transplantation all patients are at high risk of both host-versus-graft (HVG) and graft-versus-host (GVH) reactions, associated with poor graft-versus-tumour (GVT). Both GVH reactions are T-cell-mediated diseases. Given that NK cells express inhibitory receptors (KIR) for MHC class-I allotypes, a person's NK cells are capable of killing cells from individuals lacking his/her KIR epitopes. This is known as donor-versus-recipient NK cell alloreactivity. Therefore, in contrast to donor T cells, which induce HVG and GVH diseases, alloreactive donor NK cells mediates strong GVT effects against AML, improves engraftment, and increases protection from GVH disease. The combination of all these effects, associated with infusion of donor NK cells, substantially enhance the overall survival in high-risk AML patients. One suggested mechanism that can explain why NK cells not mediate severe GVH disease is that NK cells predominantly attack the haematopoietic cells of the host, in particular dendritic cells which are the main players mediating the GVH and HVG, but not other tissues which are common targets for T-cell-mediated GVH disease. Given these potential advantages of alloreactive NK cell-based therapy, donor selection for AML now involves a search for the donor who is able to mount donor-versus-recipient NK cell alloreactivity.

Other groups have investigated the transfer of professional APCs, such as macrophages and DCs. Instead of a direct antitumour effect, these cells appear to activate the immune system nonspecifically. Of interest, preclinical studies showed that when injected directly into tumour site, antigen-free DCs are able to induce tumour regression. Many of the above therapies now provide the basis for specific immunotherapeutical approaches in testing today. **See also**: Dendritic Cells (T Lymphocyte Stimulating); Macrophages; Natural Killer (NK) Cells

Specific Immunotherapy I: Adoptive Transfer

Adoptive transfer includes all therapies that involve the transfer of the actual components of the immune system already capable of directing a specific immune response. This includes both the transfer of antibodies and also specific cell types capable of, in the case of cancer therapy, mediating antigen-specific tumour regression. **See also**: Immune System

Antibody adoptive transfer

The concept of targeted therapy was conceived after exploring different mechanistic pathways contributed to the pathogenesis of malignancies, in particular the identification of the ideal target antigens. Monoclonal antibody (mAb) therapy, which acts by harnessing the host defense mechanisms, is one of innovative treatment regimens that specifically target cancer cells expressing certain receptor. There had been much interest in using antibodies in anticancer therapy. Immunologically, the antitumour effect of antibody-based therapy is believed to be the result of either complement activation and/or antibody-dependent cellular cytotoxicity. In addition, with some antibodies there may be a direct antiproliferative or apoptotic effect as a result of *cell* signalling mechanisms that occur after antibody engagement to the tumour cell. Two antibodies (rituxan and herceptin) have been approved by the FDA for use in cancer treatment (Scallon *et al*., 2006). Rituxan is specific for the CD20 antigen found on the surface of both normal and malignant B lymphocytes, and is indicated for the treatment of certain types of non-Hodgkin lymphoma (NHL). In a phase III clinical study, 50% of patients with NHL responded to rituxan. The other FDA-approved antibody, trastuzumab (herceptin), is specific for the human epidermal growth factor receptor 2 protein, HER2. HER2 is overexpressed in 25–30% of primary breast cancers and has been shown effective against this disease in phase III clinical studies. Although antibodies such as these have clear utility against cancer, there are significant limitations, the foremost being the inability to target solid tumours, which tend to be inaccessible to antibodies. Additionally, some tumours may shed their antigens into circulation, which can competitively inhibit antibodies from reaching the tumour. There are a lot of promising possibilities for the future, however, mAbs can be linked to lethal components such as radioisotopes, chemotherapeutic drugs or toxins (including diphtheria toxin or ricin), resulting in superior therapeutic approaches to treatment with mAb alone. Initial data demonstrate efficacy of single agent use, although combination therapy appears potentially more beneficial. Recent clinical studies revealed that mAb therapy is having a significant impact on several human malignancies of solid and haematological origin (Scallon *et al*., 2006). Eventually, antibody-based therapy is considered now as one of the established anticancer immunotherapeutic regimens with significant success in clinical settings. **See also**: Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC); Milstein, Cesar;

Monoclonal Antibodies: Therapeutic Uses; Non-Hodgkin Lymphomas; Tumour Antigens Recognized by Antibodies

Adoptive cell transfer (ACT)

It was noticed early in patients with cancer undergoing bone marrow transplantations that the rates of tumour remission were higher in allogenic versus autologous transplantations. At least part of this response can be attributed to the transfer of tumour specific T cells. The results of many preclinical studies have shown that the ACT of T cells can mediate tumour regression effectively. In the treatment of human cancers, ACT of tumour-infiltrating lymphocytes (TILs), concomitantly with IL-2 into patients with metastatic melanoma, can mediate tumour regression with an objective response rate of about 34% (Rosenberg, 2001). TILs are obtained by generating single-cell suspensions from tumour, and culturing these cells with IL-2. This stimulates the preferential expansion of the associated T cells. After 6–8 weeks of culture, these cells reach numbers adequate for adoptive transfer. It has been shown that the ability of the TILs to localize to the tumour site correlates positively with clinical response. Another therapeutic use of adoptive T-cell transfer is after bone marrow transplantation. Patients at high risk for developing Epstein–Barr virus (EBV)-related lymphomas can have the disease cured or prevented via infusion of EBV-specific donor T cells. **See also**: *Epstein–Barr Virus* and Cancer; Immunity: Experimental Transfer; Tumour Antigens Recognized by T Lymphocytes

There are a number of approaches investigating methods of enhancing ACT. In patients undergoing bone marrow transplant, T cells can be retrovirally modified with the herpes simplex virus thymidine kinase (HSV-TK) gene; expression of HSV-TK makes cells susceptible to the cytotoxic prodrug ganciclovir. Thus, while the patient can enjoy the potential benefits of graft-versus-tumour graft-versus-host response can be controlled by administration of ganciclovir. Another approach to improving adoptive T-cell therapy is through the transfer of tumour-specific T-cell receptor (TCR) genes. The genes encoding both chimaeric (immunoglobulin-TCR fusion) and natural TCRs have been transferred into alternate T cells and shown successfully to redirect specificity (McKee *et al*., 2005). Such modified T cells may offer a number of potential advantages, including unique or higher avidity to certain tumour antigens. Generation of such genetically modified T cells may also be less patient specific and labour-intensive, and ultimately take less time to reach the patient at a lower cost. **See also**: Herpesviruses (Human); T-cell Receptors

In all the cases, the main goal of ACT is to provide long-lasting antitumour T cells able to circulate and traffic to the tumour site in order to specifically kill the tumour cells, while sparing the normal cells to remain healthy. Early attempts of ACT therapies utilizing TILs and immunoreplete patients met with some success, however, previous preclinical studies revealed that immune ablation is an effective preconditioning regimen that can increase T-cell responses after their adoptive transfer into the hosts reviewed in Gattinoni *et al*. (2005). It is becoming increasingly clear that the state of the host environment at the time of adoptive immunotherapy can substantially impact on the functions, homing and persistence of antitumour memory T cells. In this context, recent preclinical and clinical studies revealed that induction of lymphopenia (non-myeloablative, but lymphodepleting regimen) in recipient host before adoptive transfer of T cells and vaccination is a potential approach to markedly improve the antitumour efficacy of the transferred T cells in substantial percentage ($\sim 50\%$) of patients, reviewed in Gattinoni *et al*. (2005). Of note, dramatic tumour regression can be elicited in patients with multivisceral, bulky melanoma that is refractory to standard treatments including chemotherapy, radiation and cytokine therapies. Lymphopenia can be induced by either irradiation or treatment with anticancer chemotherapeutic drug, namely cyclophosphamide and doxorubicin. Although the specific mechanisms mediating the beneficial effects of lymphopenia in this context are not fully understood (and are probably multifactorial), several mechanisms have been suggested including: (1) enhancement homeostatic expansion and trafficking of antigen-specific T cells by the creation of a niche in the immune system; (2) elimination of immunosuppressive host cells such as CD4+CD25+regulatory T cells and NKT cells; (3) the depletion of endogenous cells that compete for activating cytokines 'cellular cytokine sink hypothesis'; (4) the increased function and availability of APCs; and (5) induction of T-cell growth factors such as type I IFNs that enhance T-cell survival. Regardless of the involved mechanisms, induction of lymphopenia has been applied in clinical setting to dramatically enhance the antitumour responses against several solid tumours, in particular melanoma. Further studies aiming to understand the exact mechanisms underlying the beneficial effects of lymphopenia would substantially improve the application of this promising regimen in anticancer adoptive immunotherapy.

Specific Immunotherapy II: Vaccination

In the recent years, substantial progress has been made in vaccine development towards malignant diseases. There are sufficient data to support the notion that cancer vaccines can induce antitumour immune responses in humans with cancer. New technologies provided more information on the identification of potentially immunogenic tumour antigens that can be utilized to stimulate the patient's immune system to specifically recognize and destroy the

tumour cells. Cancer vaccination encompasses therapies that involve the administration of some form of antigen to induce a specific antitumour immune response. Although vaccination is often thought of as prophylactic in nature, most cancer vaccines are designed for therapy. Even though there is evidence that an antibody may be important in some situations leading to tumour rejection, the results of a large number of studies suggest that an appropriate T-cell component is most critical in achieving such a response. The vast majority of vaccine studies today employ measures designed to activate specific lymphocyte populations, partially because of the identification of the mechanism in which T cells can recognize antigens. While vaccine strategies are very dependent on the route of antigen delivery and adjuvant, it is important to keep in mind that the success of vaccines often depends as much on the particular antigen being used. This can be exquisitely illustrated with the human mucin tumour antigen, MUC-1, which in early vaccine studies caused cellular immune responses in mice, but humoral responses in humans. The cause for this was identified as a cross-reactive antibody normally present only in humans. It was shown, though, that vaccination conducted in a manner that allowed only for a cellular immune response, such as with a peptide-based vaccine, could overcome this problem. Thus, although MUC-1 may be somewhat of an atypical antigen, it illustrates the complexity of issues encountered when trying to use vaccination to achieve an appropriate immune response. The use of whole tumour cells or crude extracts is one of the oldest methods of cancer vaccination. Although, today, this approach is slowly being replaced by more advanced recombinant vaccines, crude tumour-based vaccines are still being tested in many trials. A common approach is to vaccinate with lethally irradiated tumour cells using an adjuvant such as BCG. Presumably, the adjuvant will create an environment in which the irradiated tumour can optimally present its tumour-associated antigen to generate an antigen-specific immune response. In a recent study of patients with stage II colonic cancer who had undergone curative resection of the primary tumour, vaccination with a tumour-BCG mixture reduced the recurrence risk by 61% (Vermorken *et al*., 1999). In another approach, studies in mice have shown that the introduction and expression of either cytokine genes (such as GM-CSF) or costimulatory genes (such as B7 or foreign MHC) may significantly improve the effectiveness of tumour cells as a vaccine. These elements are all important in creating a local environment optimally able to initiate an immune response. Still another approach is to transfer suicide genes, such as the HSV-TK gene, into tumour cells. Usually dependent on the administration of a prodrug such as ganciclovir, this can induce tumour destruction and potentially generate a systemic antitumour response. Although these approaches are advantageous in that it is not necessary to characterize tumour antigens, there are significant disadvantages. Obtaining and culturing tumour cells is difficult, and represents a time-consuming, costly and patient-specific therapy. This led to emerging of alternative approaches for cancer vaccines, including peptide- and DC-based vaccinations. In a recent systematic review with a meta-analysis of clinical studies evaluating the objective clinical and immunologic response to active specific immunotherapy in patients with colorectal cancer, pooled analysis showed an overall response rate (complete response + partial response) of 0.9% for advanced/metastatic colorectal cancer patients who received different vaccine formulations, including autologous tumour cells, peptide vaccine, DCs, idiotypic antibody and virus-based vaccine (Nagorsen and Thiel, 2006). Of these cases, humoral and cellular immune responses were in 59 and 44%, respectively, whilst minor responses and disease stabilization were described in 1.9 and 8.3% of colorectal cancer patients, respectively. Pooled results of clinical trials of active specific immunization procedures available for advanced colorectal cancer reveal a very weak clinical response rate of $<1\%$. Hence, active vaccination against weakly immunogenic tumour still require further investigation exploring novel approaches that can generate preventive clinical responses.

See also: Colon Cancer; Vaccines: Presentation; Vaccination of Humans

Virus-based vaccines

Over the last several years, advances in gene-based delivery technology have helped revitalize the field of vaccine development. This approach stemmed from an observation in 1910 of a woman who underwent remission of cervical carcinoma while receiving rabies vaccine. This was the impetus for research involving the direct injection of viruses into tumour sites, known as *in vivo* viral oncolysate vaccination. Presumably, the association of highly immunogenic viral proteins with the otherwise weakly immunogenic tumour antigens would allow for the generation of a tumour-specific immune response. Early clinical trials employing this technique, while partially successful, were plagued by inconsistency. This led to a shift to a potentially more consistent process, in which tumours were infected by virus *in vitro*. Following infection and virus-mediated cell lysis, nucleus-free cell lysate is extracted from the culture and used to vaccinate patients. The efficacy of this approach, usually with Vaccinia virus, has now been demonstrated in several cancers, including melanoma, colonic and ovarian cancers. Although there are ongoing viral oncolysate clinical trials, there has been a recent shift towards virus-based therapies that employ recombinant technology to target specific antigens. Genes encoding tumour antigens can be engineered into viral vectors, which circumvent the need for targeting the tumour. Infection of a patient with such a virus will initiate an immune response, not only against the immunogenic virus,

but also potentially against the tumour antigen. Using this approach, Vaccinia viruses expressing carcinoembryonic antigen (CEA) have demonstrated an ability to generate a CEA-specific T-cell response in cancer patients with CEA-positive tumours. A wide range of methods is now being used to try to generate a more clinically relevant immune response, including the addition of booster vaccinations and alternative viral vectors, such as adenovirus. One of the major problems with these viral vectors is that patients often have preexisting immunity to them. This may result in an inability of the viral vectors to be expressed efficiently and consequently may evoke a vector-specific rather than tumour-specific immune response. Therefore, in recent preclinical and clinical studies Schlom's group at National Cancer Institute has developed an innovative delivery system that avoids using retroviral or adenoviral delivery approaches. Their delivery system based on an avian virus vector combines delivery of the CEA gene with the three T-cell costimulatory genes (B71, ICAM-1, LFA-3, designated TRICOM). This TRICOM vector has shown the ability to induce significant improvement in antigen-specific T cell-responses and antitumour activity in clinical settings such as colon carcinoma (Marshall *et al*., 2005).

Besides using the viruses as vehicle for the antigenic domains, the viral protein itself can be a potential candidate vaccine target. For instance, cervical cancer is causally linked to human papillomavirus and constitutes a major health problem for women. This type of cancer accounts for about 10% of all cancers in women worldwide. Recently, two pharmaceutical companies, Merck and GlaxoSmithKline, have reported a remarkable degree of protection by candidate prophylactic HPV vaccines. These vaccines are based on utilizing subunit virus-like particle composed of a single viral protein, L1, which is the major structural (capsid) protein of the virus and contains the immunodominant neutralization epitopes of the virus (Lowy and Schiller, 2006). Merck uses alum as an adjuvant in its vaccine, while GlaxoSmithKline uses alum plus monophosphoryl lipid A (a detoxified form of lipopolysaccharide). Both vaccines are given as three intramuscular injections over a six-month period. **See also**: Vaccinia virus Expression System

Nonvirus-based vaccines

The viral vector-based delivery approaches of vaccine components described above although effective, it has limitations including the generation of vector-neutralizing antibodies in addition to lacking the simplicity and versatility required for universal clinical application. To minimize these disadvantages of viral-based delivery approaches, another type of vaccine approach has been developed directly based on administration of the DNA encoding a tumour antigen. First shown effective in protecting against the Influenza virus in animal studies, this approach has received growing appeal due to its practicality, safety and low cost. In general, this strategy involves putting a gene encoding a tumour antigen behind a strong promoter in a bacterial plasmid. The entire replication-deficient construct can be injected intramuscularly and, resulting in the durable expression of tumour antigens. To optimize antigen delivery as well as vaccine efficacy, other nonviral vectors have been evaluated. For example, the cationic liposome, has shown particular benefits to circumvent the obstacles that both peptide/protein- and gene-based vaccines have encountered. Liposome-mediated vaccine delivery provides greater efficacy and safer vaccine formulation for the development of vaccine for human use. The success of the liposome-based vaccine has been demonstrated in clinical trials and further human trials are also in progress. Topical vaccination has been achieved using application of naked DNA with or without tape stripping and DNA/lipid-based complex such as liposomes, polymers, transfersomes or microemulsion. All methods resulted in significant enhancement in humoral and cellular immune response over naked DNA alone (Choi *et al*., 2006).

Peptide-based vaccines

One of the most significant developments in immunology has been the understanding of T-cell recognition of processed peptides presented via MHC. In terms of tumour-associated antigens, epitopes bound directly to MHC on the cell surface can, if presented with the appropriate costimulation, activate tumour-specific T cells. As such, peptide-based vaccines can be considered, which offer numerous advantages. Administration of relevant peptides alleviates the requirement that a cell correctly processes a foreign protein. Additionally, neither tumour, virus nor other potential disease-causing agents is introduced into the cell. In MHC-matched patients, peptides can be administered as an off-the-shelf reagent as opposed to the many patient-specific requirements inherent to a tumour-based vaccine. Although it has been reported that 3 of 12 patients receiving peptide alone from the tumour-associated melanoma antigen achieved tumour regression, in general studies using peptide with or without adjuvant have shown a limited ability to stimulate a therapeutic immune response (Phan *et al*., 2003). The real potential, though, of peptide vaccination was shown in patients with metastatic melanoma who were vaccinated with a synthetic peptide–incomplete Freunds adjuvant combination followed by high dose IL-2, where 42% of patients experienced tumour regression. Current thought is that high-dose IL-2 allows the T cells activated by the peptide vaccine to expand and eliminate tumour. A number of other promising peptide-based studies are also in clinical development, including the administration of CEA-derived peptide to boost a CEA Vaccinia virus vaccine.

While the potential of peptide vaccines has been demonstrated, there are many parameters that need to be optimized, a few of which include: peptide dose, adjuvant, cytokine combination, method of delivery, optimal peptide sequences and the potential of using MHC class II and class I peptide combinations. HSPs, such as hsp70 and gp96, provide yet another potential vehicle for delivering tumour antigens into patients. These proteins, whose precise roles are not well known, appear to aid naturally in the presentation of antigen and, based on murine experiments, hold much promise as an effective adjuvant. Thus, when conjugated with tumour peptide, HSPs elicit adaptive and innate immune responses that have been tested in a variety of animal models and different human cancers. Early-phase human studies have also suggested some activity in certain cancers, and randomized phase III studies are ongoing, and these will effectively answer the question of efficacy regarding this approach to therapeutic vaccination. Conjugation of peptide with a certain antigen carrier such as keyhole limpet haemocyanin (KLH) has also been found to markedly enhance the peptide-specific immune responses. Recent studies are focusing on delivery of specific or nonspecific (viral) helper MHC calls-II epitopes at the time of MHC class-I peptide vaccination, and on vaccination with combination of MHC class-I peptides. **See also**: Antigen Recognition by Lymphocytes; Major Histocompatibility Complex: Human; Melanoma; Vaccines: Subunit

DC-based vaccines

DCs are the most potent APC and have the unique capacity to initiate primary immune responses. For clinical use, DC can be generated *in vitro* from CD34+ peripheral blood progenitor cells or monocytes. Recent work has shown that professional APCs pulsed with tumour-associated antigens *ex vivo* are capable of inducing a significant tumour-specific immune response. Loading of tumour antigens can be accomplished in a number of ways, including: (1) incubation of APCs with tumour-associated protein, (2) introduction of DNA encoding a tumour antigen into the APCs, or (3) peptide-pulsed APCs. APCs can consist of a number of different cell types, including DCs, macrophages or B cells. Of these, DCs are believed to be the cell type most adept at activating naive T cells. Unlike most tumour cells, DCs express all the molecules required for appropriate stimulation, including CD40, CD80, CD86, MHC and TLRs. A number of trials have demonstrated the potency of DCs as vehicles for delivering antigen and achieving a tumour-specific immune response. For example, in follicular B-cell lymphoma, the protein encoding a unique immunoglobulin receptor can be isolated from the tumour cells. This provides a specific idiotypic determinant, which, when given to patients via DCs can induce clinical regression. Furthermore, in a recent study, DCs pulsed with tumour-associated peptide or lysate were shown to be effective in treating metastatic melanoma, renal cell carcinoma, prostate cancer and advanced breast and ovarian cancers (Brossart, 2002). DCs are also considered as a potential target for gene therapy, where they are thought to play at least three distinct roles: (1) MHC class II-restricted presentation of antigens secreted by neighbouring, transfected cells, (2) MHC class I-restricted 'cross' presentation of antigens released by neighbouring, transfected cells, and (3) direct presentation of antigens by transfected DC themselves. Notably, ample evidence from recent studies affirms that stimulation of DCs by TLRLs either *in vitro* or *in vivo* induces their full activation and differentiation into functional APCs. Therefore, current clinical studies are focusing on vaccination with TLRL-conditioned DCs loaded with different forms of tumour antigens. **See also**: Antigen-presenting Cells

Idiotypic antibody-based vaccines

While the vast majority of cancer vaccines are directed at achieving a cellular immune response, there are now a number of different strategies aimed at achieving a humoral immune response. Several studies have shown a correlation between tumour regression and presence of tumour-specific antibodies. Although a number of potential targets have been identified, including Her2-neu, CEA and P53, the carbohydrate antigens, specifically the gangliosides, may represent the most promising target for vaccine-generated antibodies. Gangliosides, which are neuraminic acid-containing glycosphingolipids present on the cell membrane, are often overexpressed in melanomas, sarcomas and some other types of tumours. In an early vaccine trial, it was demonstrated that vaccination with GM2 (a ganglioside) and BCG resulted in a 14% increase in overall survival versus BCG alone in patients with metastatic melanoma without preexisting antibodies against GM2 (Livingston *et al*., 1994). Current trials are now experimenting with other adjuvants, such as KLH, as well as alternative gangliosides including GD2, GD3 and 9-*O*-acetyl GD3. Idiotypic vaccines offer yet another humorally directed method of vaccination. Generally, this involves the creation of an antibody that mimics a natural tumour antigen. The body can respond to the idiotypic antibody with a humoral response and, potentially, a subsequent cellular immune response. A number of idiotypic antibodies have shown the ability to generate an immune response, including the 3H1 antibody, which mimics the CEA antigen. In a phase 1 trial, administration of this antibody was shown to improve survival in patients with advanced colorectal cancer to a level comparable with that of several chemotherapeutic agents. While humorally directed vaccines represent an alternative, the mainstream approach is initially to activate the cellular branch of the

immune system. **See also**: Idiotypes; Immunity: Humoral and Cellular

Conclusion

There are an increasing number of novel and promising approaches in tumour immunotherapy. These have yet to be of clinical benefit to the majority of patients with cancer; however, given the complexity of the immune system and its interaction with tumours, significant progress has been made. In metastatic melanoma, a disease usually fatal within 6 months of diagnosis, objective regression can now be obtained in over one-third of patients by either adoptive T-cell transfer or vaccination. In many other cancers, including renal cell carcinoma, colonic cancer, bladder cancer and many leukaemias, there are important new therapies that have been developed only in the past decade. These advances have correlated with improvements in recombinant technology as well as advanced tumour immunology. There are also many promising approaches for the future, such as combined therapies, in particular lymphoablation, adoptive T-cell transfer, and cytokine regimens. It will also probably be of increasing benefit to combine more conventional treatment options with immunotherapy, in particular nonspecific approaches concomitant with vaccination or adoptive T-cell therapy. Ultimately, though, perhaps the biggest gains in immunotherapy may be in prophylactic therapy, where with a healthy individual it may be easier to generate a fully protective immune response.

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