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REVIEW

Trends in Cancer Immunotherapy

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Abstract: Modulation of the immune system for therapeutic ends has a long history, stretching back to Edward Jenner's use of cowpox to induce immunity to smallpox in 1796. Since then, immunotherapy, in the form of prophylactic and therapeutic vaccines, has enabled doctors to treat and prevent a variety of infectious diseases, including cholera, poliomyelitis, diphtheria, measles and mumps. Immunotherapy is now increasingly being applied to oncology. Cancer immunotherapy attempts to harness the power and specificity of the immune system for the treatment of malignancy. Although cancer cells are less immunogenic than pathogens, the immune system is capable of recognizing and eliminating tumor cells. However, tumors frequently interfere with the development and function of immune responses. Thus, the challenge for cancer immunotherapy is to apply advances in cellular and molecular immunology and develop strategies that effectively and safely augment antitumor responses.

Keywords: Cancer, immunotherapy, vaccines, antibodies, peptides, cytokines, clinical trials

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Introduction

Advances in cellular and molecular immunology over the past three decades have provided enormous insights into the nature and consequences of interactions between tumors and immune cells. This knowledge continues to lead to strategies by which the immune system might be harnessed for therapy of established malignancies.

Cells of the innate immune system respond to "danger" signals, which can be provided by growing tumors as a consequence of the genotoxic stress of cell transformation and disruption of the surrounding microenvironment. Under ideal conditions, these signals induce inflammation, activate innate effector cells with antitumor activity, and stimulate professional antigen-presenting cells (APCs), particularly dendritic cells (DCs), to engulf tumor-derived antigens and migrate to draining lymph nodes to trigger an adaptive response by T and B lymphocytes. Despite this well-orchestrated surveillance operation, the presence of a tumor indicates that the developing cancer was able to avoid detection or to escape or overwhelm the immune response. Progressing tumors often exhibit strategies that promote evasion from immune recognition.[1] This includes physical exclusion of immune cells from tumor sites, poor immunogenicity due to reduced expression of major histocompatability complex (MHC) or co-stimulatory proteins, and disruption of natural killer (NK) and natural killer T (NKT) cell recognition.[2] Additionally, some tumors prevent triggering of an inflammatory response by secreting proteins, such as interleukin (IL)-10 or vascular endothelial growth factor (VEGF) that interfere with DC activation and differentiation[3] or by blocking the production of pro-inflammatory molecules by increasing expression of the STAT3 protein.[4] Even if a response is induced, tumor cells may escape elimination by losing targeted antigens, rendering tumor-reactive T-cells anergic, inducing regulatory T-cells, or specifically deleting responding T-cells.[5,6] Thus, there is often a cat and mouse game with the immune system exerting pressure to eliminate the tumor, and the tumor cells evading the immune response; the eventual tumor that develops reflects "immunoediting" with selection of poorly immunogenic and/or immune-resistant malignant cells.[7] Despite these obstacles, modern immune-based therapies continue to show increased potential for treating malignant diseases.

Innate Cells as Initiators of the Adaptive Immune Response

One of the first strategies to enhance immune responses to cancer was the administration of adjuvants directly into solid tumors to stimulate inflammation and recruit immune effector cells. This approach is still commonly used for treating superficial bladder carcinomas and has been used to treat melanoma and neurological tumors. It is now known that many of these adjuvants contain bacterial products, such as lipopolysaccharide (LPS) or CpG-containing oligo-deoxynucleotides recognized by toll-like receptors (TLRs) on innate immune cells. This leads to the production of pro-inflammatory cytokines and facilitating productive interactions between the innate and adaptive immune responses.[8] Although many tumors render this strategy ineffective by producing proteins, such as transforming growth factor (TGF)-β to prevent activation of the immune response,[9] more recent reports describes $CD8^+$ help for innate antitumor immunity[10] and cooperative action of CD8 T lymphocytes and natural killer cells controlling tumor growth under conditions of restricted T-cell receptor diversity.[11]

Several papers have also described the role of adaptive immunity not only in suppressing but also activating innate immune responses in other diseases. These include the role of $CD8^+$ T cells mediating antibacterial immunity via CCL3 activation of TNF/ROI^+ phagocytes[12] or contributing to macrophage recruitment and adipose tissue inflammation in obesity.[13] Furthermore, studies investigating cooperation between innate and adoptive immunity cooperating flexibly to maintain host-microbiota mutualism[14] or dampening of innate immune responses by T cells through inhibition of NLRP1 and NLRP3 inflammasomes,[15] have also been described.

Cellular Immunotherapy

T-cells express clonally distributed antigen receptors that in the context of MHC proteins can recognize either unique tumor antigens, those evolving from mutations or viral oncogenesis or self-antigens, those derived from over expression of proteins or aberrant expression of antigens that are normally developmentally or

tissue-restricted. To mediate antitumor activity, T-cells must first be activated by bone marrow—derived APCs that present tumor antigens and provide essential co-stimulatory signals,[16] migrate and gain access to the tumor microenvironment, and overcome obstacles to effective triggering posed by the tumor. Activation results in the production of cytokines, such as interferon (IFN) and tumor necrosis factor (TNF) that can arrest proliferation of malignant cells and prevent the angiogenesis necessary for tumor growth, and also lysis of tumor cells mediated by perforin and/or Fas. Consequently, efforts have focused on identifying tumor antigens, providing the antigens in immunogenic formats to induce responses, manipulating T-cell responses to increase the number of reactive cells and augmenting effector functions (Table 1).

Active and Passive Immunotherapy

A number of immunologic interventions, which can be divided into both passive and active, can be directed against tumor cells.[17] In passive cellular immunotherapy, specific effector cells are directly infused and are not induced or expanded within the patient. Lymphokine-activated killer (LAK) cells are produced from the patient's endogenous T cells, which are extracted and grown in a cell culture system by exposing them to interlukin-2 (IL-2). The proliferated LAK cells are then returned to the patient's bloodstream. Clinical trials of LAK cells in humans are ongoing. Tumor-infiltrating lymphocytes (TILs) may have greater tumoricidal activity than LAK cells. These cells are grown in culture in a manner similar to LAK cells. However, the progenitor cells consist of T cells that are isolated from resected tumor tissue. This process theoretically provides a line of T cells that has greater tumor specificity than those obtained from the bloodstream. Concomitant use of interferon enhances the expression of major histocompatability complex (MHC) antigens and tumor-associated antigens (TAAs) on tumor cells, thereby augmenting the killing of tumor cells by the infused effector cells.

Active immunotherapy

Inducing cellular immunity (involving cytotoxic T cells) in a host that failed to spontaneously develop an effective response generally involves methods to enhance presentation of tumor antigens to host effector cells. Cellular immunity can be induced to specific, very well-defined antigens. Several techniques can be used to stimulate a host response; these may involve giving peptides, DNA, or tumor cells (from the host or another patient). Peptides and DNA are often given using antigen-presenting cells (dendritic cells). These dendritic cells can also be genetically modified to secrete additional immune-response stimulants (e.g. granulocyte-macrophage colony-stimulating factor (GM-CSF) that will be discussed in more detail later.

Nonspecific immunotherapy

Interferons (IFN-α, -β, -γ) are glycoproteins that have antitumor and antiviral activity. Depending on dose, interferons may either enhance or decrease cellular and humoral immune functions. Interferons also inhibit division and certain synthetic processes in a variety of cells. Clinical trials have indicated that interferons have antitumor activity in various cancers, including hairy cell leukemia, chronic myelocytic leukemia, AIDS-associated Kaposi's sarcoma, non-Hodgkin lymphoma, multiple myeloma, and ovarian carcinoma. However, interferons may have significant adverse effects, such as fever, malaise, leukopenia, alopecia, and myalgias.

Adoptive Immunotherapy

High-dose chemo-radiotherapy followed by rescue from the resulting ablation of normal bone marrow with an allogeneic hematopoietic stem cell transplant (HSCT) has become standard therapy for many hematologic malignancies. One problem with this treatment is graft-versus-host disease (GVHD), due to allogeneic donor-derived T-cells injuring the "foreign" normal tissues of the host. However, malignant cells that survive chemoradiotherapy are also of host origin, and patients who develop GVHD have lower relapse rates from an associated graft-versus-tumor (GVT) effect. T-cells mediate this antitumor activity, as affirmed by the complete responses sometimes observed in patients who receive infusions of donor T-cells to treat relapse after HSCT and in recipients of a newly developed non-myeloablative allogeneic HSCT regimen in whom, because of the absence of high-dose chemoradiotherapy, all antitumor effects must result from GVT effects.[18] However, the GVT activity with these regimens is often associated with

severe and life-threatening GVHD. Ongoing efforts to define antigenic targets with limited tissue distribution, permitting donor lymphocytes to preferentially target malignant cells and not critical normal tissues, coupled with methods to generate and/or select T-cells with such specificities, should provide a much-needed refinement to this approach.[19]

An alternative to using allogeneic T-cells to mediate antitumor responses has been to isolate autologous tumor-reactive T-cells, expand the cells *in vitro*, and then re-infuse the cells back into the patient. This approach circumvents many of the obstacles to generating an adequate response *in vivo*, as the nature of the APCs and components of the microenvironment can be more precisely controlled *in vitro*. However, this strategy has required the recent development of methods to extensively manipulate T-cells *in vitro* with retention of specificity and function, such that after infusion the cells will survive and migrate to and eliminate tumor cells.

Initial therapies used tumor-infiltrating lymphocytes as an enriched source of tumor-reactive cells, but such cells can also usually be obtained from circulating blood lymphocytes. Although optimal methods for stimulating and expanding antigen-specific T-cells *in vitro* are still being defined, in general, DCs presenting the antigen are used to initially trigger reactive T-cells, which can then be selected and stimulated with antibodies to CD3. Supplemental cytokines are provided during cell culture to support lymphocyte proliferation, survival, and differentiation. With this approach, it has been possible to expand tumor-reactive T-cells to enormous numbers *in vitro*, infuse billions of specific cells without overt toxicity to achieve *in vivo* frequencies beyond that attainable with current vaccine regimens. However, despite the high *in vivo* frequencies of tumor-reactive effector cells achieved, only a fraction of patients respond, indicating the existence of additional hurdles. One essential requirement is that infused cells must persist to mediate an effective response. Analogous adoptive therapy trials for cytomegalovirus and Epstein-Barr virus infection in immuno-suppressed hosts have demonstrated increased *in vivo* proliferation and persistence of CD8 effector T-cells in the presence of specific CD4 helper T-cells.[20] Such CD4 T-cells likely provide many beneficial functions, including cytokine production and APC activation, which can improve the quality and quantity of the CD8 responses, as well as direct effector activities against infected or tumor targets. However, unlike viral responses that induce robust CD4 and CD8 responses, identifying and characterizing the specificity of tumor-reactive CD4 T-cells has proven considerably more difficult than with CD8 responses. Additionally, obstacles to safely maintaining a CD4 response reactive with a potentially normal protein remain to be elucidated. Consequently, CD4 help is largely provided to transferred tumor-reactive CD8 cells in the form of surrogate exogenous cytokines. The largest experience is with IL-2, which prolongs persistence and enhances the antitumor activity of transferred CD8 cells.[21] Alternative cytokines such as IL-15, IL-7, and IL-21, as well as activation of APCs with antibodies to CD40, are currently being evaluated in preclinical studies.

Although polyclonal infusion has shown promising outcomes in some tumor models that are susceptible to antigenic drift or loss of immune selection,[22,23] the infusion of T-cell clones represents an appealing refinement of adoptive therapy because the specificity, avidity, and effector functions of infused cells can be precisely defined. This facilitates subsequent analysis of requirements for efficacy, basis for toxicity, and rational design of improved therapies. The transfer of antigen-specific CD8 T-cell clones has been shown to be effective for prevention of viral infections and treatment of malignant disease.[25] Such studies have also formally demonstrated that low, nontoxic doses of IL-2 are sufficient to promote the *in vivo* persistence and antitumor activity of CD8 T-cells.

Cancer Vaccines

Therapeutic cancer vaccines target the cellular arm of the immune system to initiate a cytotoxic T-lymphocyte response against tumor-associated antigens.[24] The development of human therapeutic cancer vaccines has come a long way since the discovery of major histocompatability complex (MHC) restricted tumor antigens in the eighties. The simplest model of immune cell-mediated antigen-specific tumor rejection consists of three elements: appropriate antigen, specific for the tumor, efficient antigen presentation and the generation of potent effector cells. Moreover, the critical time when immune responses against the tumor are most important should also be determined. While eliminating some early transformed cells may be ongoing in an

asymptomatic way as part of the immunosurveillance, if early elimination failed, equilibrium between small tumors and the immune system may be established. If the immune system is unable to maintain this equilibrium, tumors may escape and it is this last phase when they become symptomatic. Therapeutic cancer vaccines are applied in this last phase in order to reverse the lack of tumor control by the immune system. In addition to the increasing knowledge about how to optimize the elements of anti-tumor immunity in order to generate clinically relevant responses, there is an ever-increasing list of immune evasion mechanisms impeding the efforts of cancer vaccines. This indicates that the elements necessary for immune-mediated tumor rejection need to be optimized.[25]

Potential tumor associated antigens (TAAs) can be identified by the elution of peptides from MHC molecules on tumor cells,[26] or with proteomic approaches such as 2-dimensional gel electrophoresis, MALDI-MS and SELDI-MS (matrix-assisted or surface enhanced laser-desorption ionization mass spectrometry).[27] Serological analysis of recombinant cDNA expression libraries (SEREX) is another widely used method; it utilizes sera of cancer patients to detect over expressed antigens from tumor cDNA libraries.[28] Furthermore, several RNA-based methods have also gained importance; transcriptome analysis that include DNA microarrays,[29] serial analysis of gene expression (SAGE),[30] comparative genomic hybridization (CGH)[31] and massively parallel signature sequencing (MPSS).[32] These methods provide an enormous amount of information and require complex computer-aided analysis and interpretation of the data, referred to as gene expression profiling. This is necessary in order to find gene expression patterns and to distinguish them from noise.[33]

Following promising *in vitro* immunogenicity studies,[34] multicentre vaccine trials have been organized with the sponsorship of the Cancer Vaccine Collaborative (NCI and Ludwig Institute for Cancer Research). These trials have provided some information about the optimum route of administration, type of vaccine, type of adjuvant, endpoints, etc.[35] When testing the immunogenicity of candidate antigens and defining epitopes, it should be remembered that T-cells with high avidity for self antigen undergo negative selection during T-cell development, thus the new TAAs may only generate T-cell responses of intermediate or low affinity. Furthermore, the wide range of restriction elements in the human population means that due to the combination of tolerance and immunodominance, potentially ideal TAAs will not be equally immunogenic in all patients. Antigen loss may also occur during tumor progression, as TAAs which are not necessary for the maintenance of the transformed phenotype may be deleted and tumor cells in advanced disease may express antigens different from those in early stages.[36]

Dendritic cells

DCs are the main antigen presenting cells in the body[37] and their generation for anti-tumor immunity has been the focus of a vast array of scientific and clinical studies.[38] Immature DC (iDC) patrol the peripheral tissues, sampling antigen from the environment. Following their activation, DC undergo a maturation process that involves the upregulation of T cell co-stimulatory molecules, (e.g. CD80, CD86), increased cytokine secretion, a transient increase in phagocytosis followed by reduced antigen uptake and expression of migratory molecules such as CCR7. These changes equip mature DC (mDC) to prime naïve T cells in the lymph nodes, in contrast to iDC that induce T cell tolerance to antigen.[39]

The ability of DCs to present protein tumor antigens (T-Ags) to $CD4^+$ and $CD8^+$ T-cells is pivotal to the success of therapeutic cancer vaccines. DC's specialized capacity to cross-present exogenous Ags onto major histocompatability (MHC) class I molecules for the generation of T-Ag-specific cytotoxic T lymphocytes (CTLs) has made these cells the focal point of vaccine-based immunotherapy of cancer.

DC can be loaded exogenously with TAA using whole cell populations or short peptides corresponding to epitopes from specific TAA. Whilst the use of DC pulsed with short peptides can yield information on immune activation following therapy, they are not ideal therapeutic agents for a number of reasons. The most obvious reason is the use of specific TAA depends on the identification of relevant TAA and not all cancers have well defined TAA. Moreover, TAA expression within a tumor can be very heterogeneous[40] thus priming CTL specific for defined TAA peptides may encourage the outgrowth of non-expressing clones, leading to immune evasion. Furthermore, both MHC-1 and MHC-II epitopes are required for efficient

T cell priming. While a number of MHC-1 restricted peptides have been identified, fewer MHC-II epitopes are known. Synthetic long peptides, comprising both MHC-I and MHC-II epitopes, which require processing by DC before presentation, can overcome some of the limitations of small peptides, as they lead to extended epitope presentation. An alternative to pulsing with peptide epitopes is to load DC with whole tumor cell preparations in the form of lysates, whole dead cells or by fusing DC with tumor cells.[41] Both allogeneic and autologous tumor material has been used to load DC with clinical trials carried out using preparations using both types.[42]

Genetic modification of DC, using recombinant DNA viruses encoding TAA, has been demonstrated by several groups, and can enhance T cell priming potential via antigen presentation. DC transduced to express the model tumor antigen β-galactisidase, using a recombinant adenoviral vector, were able to generate antigen-specific CTL responses.[43] A phase I/II trial using genetically modified DC, showed that autologous DC could be transduced with high efficiency using a replication-defective adenovirus expressing full length melanoma-associated antigen recognized by T-cells (MART-1), and that the DC processed and presented the antigen for at least 10 days. Evidence of MART-1 specific $CD4^+$ and $CD8^+$ responses were found in around 50% of patients following vaccination.[44]

In addition to loading DC with antigen, genetic approaches have been used to further optimize the maturation state of DC, for example, DC transfected with GM-CSF demonstrated increased antigen presentation and better migratory capacity, which translated into enhanced immune priming *in vivo*.[45] Other approaches include genetically modifying DC using adenoviral or retroviral vectors to directly express TH1 cytokine IL-12,[46] an adenovirus encoding CD40 L[47] and modifying DC to express co-stimulatory molecules CD40 L, CD70 and TLR4 called "TriMix",[48] and heat shock protein.[49] Furthermore, vaccines coupled to TLR ligands lead to efficient CTl activation by endogenous DC[50] and the use of oncolytic viruses also looks particularly promising.[51]

Treg cells

Since their discovery in the 1960s as suppressive T cells, Tregs have been extensively studied in a wide range of both physiological and pathological conditions in man.[52] Treg suppress T-cell responses and provide another mechanism compromising the development of effective tumor immunity.[53] These cells are usually $CD4^+$ and are distinguishable phenotypically by expression of CD25 (the chain of the IL-2 receptor required for high affinity binding), high levels of CTLA-4, the glucocorticoid-induced TNF-related receptor (GITR), and the forkhead transcription factor Foxp3. Expression of TNFR2 defines a maximally suppressive subset of mouse $CD4^+CD25^+FoxP3^+$ Treg[54] and co-expression of TNFR2 and CD25 identifies more of the functional $CD4^+FoxP3^+$ regulatory Treg cells in peripheral human blood.[55]

Treg cells can arise in response to persistent antigen stimulation in the absence of inflammatory signals, particularly in the presence of TGF-ß, and have been detected in increased frequency in some cancer patients. Furthermore, tumor-induced expansion of regulatory T cells by conversion of $CD4^+CD25^+$ lymphocytes is thymus and proliferation independent.[56] Thymosin alpha1 is a peptide with a multitude of effects in the organism both from its direct influence on the cells, as well as modulation of the immune system.[57] When administered *in vivo* it strengthens the immune reaction in a whole variety of animal models and its optimal reaction occurs in coordination with other agents.[58]

Inhibiting Treg cell function in patients with cancer is an essential step if new therapies, especially immunotherapies, are to be clinically successful. Initial studies have indicated that depleting Treg cells from cancer patients might be a valid approach; more recent preliminary data has raised the hypothesis that functionally inactivating Treg cells might be a better alternative. Studies in murine tumor models targeting all $CD25^+$ T-cells for depletion have appeared promising.[59] However, activated effector CD8 and CD4 T-cells also express CD25, and depletion of these cells during the acute phase of the antitumor T-cell response may severely limit the application of this approach. The availability of the anti-CD25 monoclonal antibody, PC61, has enabled the effects of Treg cell depletion to be tested in murine models.[60] Despite some efficacy, intrinsic limitations apply when PC61 is used to treat established tumors as time course experiments have reported that its efficacy is lost as tumors progress.[61] Other monoclonal antibodies to human CD25 that are available for clinical use, such as daclizumab,

block IL-2 and receptor interactions are used to treat hematologic malignancies.[62] However, to date, most studies in humans have used the immunotoxin denileukin difitox (Ontak), a fusion protein between the IL-2 and diphtheria toxin, to selectively kill lymphocytes expressing the IL-2 receptor. The *in vivo* anti-tumor efficacy is still under preclinical and clinical investigation with discrepant results reported so far.

Another approach is to inhibit tumor-specific Treg cell expansion. This could be achieved by inhibiting the indoleamine 2, 3-dioxygenase (IDO) pathway. Preclinical data confirm that the administration of an IDO inhibitor significantly decreases the rate of peripheral conversion and dramatically impairs tumor growth.[63] Another possible target is transformed growth factor (TGF), involved in both proliferation and conversion of Treg cells in tumor bearers. Genetically engineered mice express a dominant negative form of the TGF receptor on lymphocytes show reduced, if not absent, growth of several transplanted tumors.[64] Moreover, CTLA-4 blockade or GITR triggering has been shown to reverse immune suppression as a result of Treg function both *in vitro* and *in vivo*.[65] Ultimately, by inducing Treg expansion, the tumor takes advantage of the inhibitory function that these cells exert on all the immune components. Avoiding the physical elimination of Treg cells would be potentially useful as it would prevent the induction of a new wave of peripherally converted Treg cells that are endowed with a wide TCR repertoire. Conversion would also redirect potential effector T cells toward the Treg cell phenotype. Alternatively, Treg cell inactivation is a suitable strategy, which would functionally impair Treg cell suppression without changing the TCR repertoire of the expanded Treg cell population. Triggering of TLR8 or OX40, and potentially blocking adenosine, might improve the chances of neutralizing Treg cell immunosuppression in cancer immunotherapy.

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells that expand during cancer, inflammation and infection, and have a remarkable ability to suppress T-cell responses.[66] Although suppressive myeloid cells were described more than 20 years ago in patients with cancer,[67] their functional importance in the immune system has only recently been appreciated.

Accumulating evidence has now shown that that this population of cells contributes to the negative regulation of immune responses during cancer and other diseases. Common features to all MDSCs are their myeloid origin, their immature state and a remarkable ability to suppress T-cell responses. In addition to their suppressive effects on adaptive immune responses, MDSCs have also been reported to regulate innate immune responses by modulating the cytokine production of macrophages.[68] More recently, it has become clear that the suppressive activity of MDSCs requires not only factors that promote their expansion, but also factors that induce activation. The expression of these factors, which are produced mainly by activated T cells and tumor stromal cells, is induced by different bacterial and viral products, or as a result of tumor cell death.[69]

Macrophages

Macrophages undergo activation in response to environmental signals, including microbial products and cytokines.[70] In response to some bacterial moieties e.g. lipopolysaccharide LPS) and IFN-γ, macrophages undergo classic (M1) activation. Alternative (M2)-activated macrophages come in different varieties depending on the eliciting signals mediated through receptors that include IL-4, IL-13, immune complexes plus signals mediated through receptors that involve downstream signaling through MyD88, glucocorticoid hormones and IL-10. The various forms of M2 activation are oriented to the promotion of tissue remodeling and angiogenesis, parasite encapsulation, regulation of immune responses as well as promotion of tumor growth. Recent results have highlighted the integration of M2-polarised macrophages with immunostimulatory pathways. They have been shown to induce differentiation of Treg cells[71] and conversely, Tregs have been reported to induce alternative activation of human mononuclear phagocytes.[72] Cancer has thus served as a paradigm of *in vivo* M2 polarization.[73]

Physical Barriers, Tumor Stroma and Vessels

The tumor environment represents another challenge for cancer vaccines. Established epithelial tumors can be surrounded by basal-membrane-like structures which prevent infiltration by lymphocytes and the expansion of tumor-specific T-cells at the tumor site and in lymphoid tissues.[74] Solid tumors larger than about 1–2 mm

in diameter require the presence and support of stromal cells for blood supply, growth factors and structural support. The stroma consists of cancer-associated fibroblasts (CAF), tumor endothelial cells (TEC) and tumor-associated macrophages (TAM) and can represent more than 50% of the tumor tissue depending on the type tumor.[75] Stromal cells do not only represent a physical barrier but also release soluble mediators (TGF-β, IL-10, prostaglandin) which inhibit immune responses and promote angiogenesis and tumor progression.[76,77] Conventional cancer treatments, such as de-bulking surgery, chemo- or radiotherapy, not only destroy tumor cells but also destroy or damage stromal cells that may contribute to breaking immunological resistance and immunosuppression.[78] The intricate interplay between tumor and stroma attracts their simultaneous immune destruction: when highly expressed TAAs on tumor cells are cross-presented by stromal cells to T-cells, the stromal component also becomes a target of cytotoxic T-cell killing.[79]

TGFβ-1 regulates the production of cytokines and growth factors by stromal and tumor cells, such as fibroblast growth factor (FGF), connective tissue growth factor (CTGF) and vascular endothelial growth factor (VEGF), which promote angiogenesis and tumor progression.[45] The new tumor vasculature is generally both structurally and functionally abnormal, which makes trafficking/recirculation of the tumor tissue by lymphocytes and treatments including cancer vaccines, extremely difficult. Anti-angiogenic treatments, including immunological targeting of antigens over-expressed on endothelial cells during angiogenesis or antibody blockade of VEGF-receptors "normalize" the tumor vasculature.[80,81] This treatment also reverts epithelial tumors to non-invasive type and may also aid the penetration of vaccines and other treatments in the tumor tissue. Moreover, IL-12 inhibits angiogenesis via an IFN-γ mediated pathway,[82] while adoptively transferred tumor-specific $CD8^+$ T-cells destroy the vasculature of established tumors via an antigen-independent, IFN-γ-dependent mechanism.[83]

Mechanisms of Tumor Induced Tolerance/Escape from the Immune System

Despite the evidence that immune effectors play a significant role in controlling role in tumor growth under natural conditions or in response to therapeutic manipulation, it is well known that malignant cells can evade immune surveillance.[84] This is due in part to the fact peptides with sufficient immunogenic potential are not presented by malignant cells to antigen presenting cells under molecular/cellular conditions conducive to an effective immune response. From a Darwinian perspective, the neoplastic tissue can be envisaged as a microenvironment that selects for better growth and resistance to the immune attack. Cancer cells are genetically unstable and can lose their antigens by mutation. This instability, combined with an immunological pressure, could allow for selective growth of antigen-loss mutants.[85] Mechanistically this could operate at several levels including: loss of the whole protein or changes in immunodominant T-cell epitopes that alter T-cell recognition, antigen processing or binding to the MHC. Antigen loss has been demonstrated in patients with melanoma and B-cell lymphoproliferative disease.[86,87] Moreover, many cancer vaccines aim to induce a therapeutic $CD8^+$ cytotoxic T-cell response against TAAs. This in turn is dependent on correct processing and presentation of TAAs by MHC class I molecules on tumor cells. This pathway is complex and involves multiple intracellular components. Defects in the components of the MHC class I antigen processing pathway are frequently found in human cancers and can occur in concert with the loss of tumor antigens.[88,89] Other cancer related mechanisms underlying tumor immune escape include loss of TAA expression,[90] lack of co-stimulatory molecules expression,[91] inactivating mutations of antigen presentation related molecules,[92] production of soluble immunosuppressive factors such as transforming growth factor beta (TGF-beta), interlukin-10 (IL-10), reactive oxygen species (ROS), nitric oxide (NO), produced by tumor cells.

Candidates for Immunotherapy in Oncology

Malignant melanoma, renal cancer and prostate cancer are potentially immunogenic, making them good candidates for immunotherapeutic approaches.[93,94] Melanoma has been the most popular target for T-cell-based immunotherapy in part as it is much easier to grow tumor-reactive T-cells from melanoma patients than any other type of human cancer.[95] However, many promising immune-based therapies have been ineffective in human clinical trials.[96] For example, although

IL-2, licensed for use in malignant melanoma in the USA, can induce long-term regression of metastatic tumors it has been associated with high levels of toxicity.[97] As yet, no approved therapy for advanced melanoma has improved overall survival to date. Other immunotherapies for melanoma have not been used outside the setting of clinical trials.

Immunotherapeutic approaches currently under investigation for renal cancer include vaccines, which have been used with limited success. In a Phase I trial, a granulocyte-macrophage colony-stimulating factor (GMCSF)-secreting vaccine administered to patients with metastatic renal cancer induced significant tumor regression in one patient. Additionally, infusion with lymphocytes that secrete anti-tumor cytokines, such as tumor necrosis factor, has also been used in clinical trials.[98]

IL-2 is approved in the USA for the adjuvant therapy of stage III renal cancer.[99] In some cases IL-2 has been demonstrated to induce long-term regression of metastatic tumors and durable complete responses of metastatic tumors, probably by inducing T-cell activation. Interferon-α has been used in clinical trials and has demonstrated a response rate of 15%–20% in patients with metastatic disease. Combination therapy with IL-2 has demonstrated improved response rates versus IFN-α alone, although this has not been shown consistently.[62]

Combination Immunotherapy

A deeper understanding of the mechanisms underlying the generation of tumor immunity has provided a framework for developing more potent immunotherapies. A major insight is that combinatorial approaches that address the multiplicity of defects in the host response are likely to be required for clinical efficacy.[100] In addition to surgery, nanotechnology[101] and molecular imaging[102] are methods employed with cancer immunotherapy. The following summarizes some of the combinations that have been tested in laboratory and clinical settings.

Chemotherapy and mAb

Immunostimulatory mAbs directed to immune receptors have emerged as a new and promising strategy to fight cancer. In general, mAbs can be designed to bind molecules on the surface of lymphocytes or antigen presenting cells to provide activating signals e.g. CD28, CD137, CD40 and OX40.[103] Mabs can also be used to block the action of surface receptors that normally down regulate immune responses, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and PD-1/B7-H1. In combined regimes of immunotherapy, these mAbs are expected to improve therapeutic immunizations against tumors as observed in preclinical studies. Anti-4–1BB (agonistic anti-CD137) mAb has been successfully tested as an anti-cancer molecule in pre-clinical studies.[104] Clinical trials of chemotherapy and mAb have resulted in some efficacy against cancer in patients.[105] For example, tremelimunamab induced durable objective responses with low-grade toxicities when used as second-line monotherapy in a phase-I study with melanoma patients treated with single, escalating doses.[106] Moreover, phase I studies of ipilimumab were performed in patients with prostate, melanoma and ovarian cancer. In these studies, patients after a single administration of ipilimumab achieved some clinical efficacy as demonstrated by incomplete reduction of tumor size with extensive tumor necrosis with leukocyte infiltration. In phase II studies, repeated administrations with ipilimumab allowed more patients to achieve objective responses.[107] The combination of ipilimumab with chemotherapeutics (dacarbazine)[108] or docetaxel,[109] with IL-2[110] or with melanoma-associated peptide vaccines[111] improved the rate of complete responses in patients compared with the monotherapy arms.

Chemotherapy and active specific immunotherapy

Clinical trials utilizing both chemotherapy and vaccine therapy have been performed in patients with different cancer types, including glioblastoma multiforme (GBM),[112] colon cancer,[113] pancreatic cancer,[114] prostate cancer[115] and small cell lung cancer.[116] For example, Wheeler et al (2004)[112] investigated the clinical responsiveness of GBM to chemotherapy after vaccination. Three groups of patients were treated with chemotherapy alone, vaccination alone or chemotherapy after vaccination. All patients subsequently underwent a craniotomy and received radiation. The vaccination consisted of autologous dendritic cells loaded with either peptides from cultured tumor cells or autologous tumor lysate. Results demonstrated a significantly longer post chemotherapy survival in the vaccine/chemotherapy group when

compared with the vaccine and chemotherapy groups in isolation. Overall, data suggests that vaccination against cancer-specific antigens can sensitize the tumor against subsequent chemotherapeutic treatment. Although the mechanisms that underlie such a synergistic effect have not yet been elucidated, it is speculated that the vaccination-induced increase in the frequency of primed T cells constitutes a major advantage by the time the tumor microenvironment is modified by cytotoxic drugs.

Chemotherapy and adoptive lymphocyte immunotherapy

Lymphodepletion by chemotherapy followed by the adoptive transfer of lymphocytes has been evaluated in small scale studies in melanoma patients.[117] In a study by Dudley et al 2005,[118] 35 patients were adoptively transferred with autologous cytotoxic lymphocytes with the administration of IL-2 1 day after cyclophosphamide and pludarabine administration. They observed a complete response in only 3 patients, partial responses in 15 and no response in 17 patients. Larger-scale studies are needed to assess the efficacy of this treatment modality in cancer patients.

Humoral Immunotherapy

B-cell activation results in the production of antibodies that can bind to immunogenic cell-surface proteins on tumor cells. These initiate complement-mediated cell lysis, bridge NK cells or macrophages to the tumor for antibody-dependent T-cell-mediated cytotoxicity (ADCC). They in turn interfere with tumor cell growth by blocking survival or inducing apoptotic signals, or increase immunogenicity by facilitating the uptake and presentation of tumor antigens by APCs. Thus, enhancing B-cell responses *in vivo* or providing a large amount of *in vitro*—generated antibodies has the potential to promote antitumor activity.

The widely used, rituximab, binds CD20 and if given alone or with chemotherapy, can induce high rates of remission in patients with B-cell lymphomas,[119] as does cetuximab, which completely inhibits the binding of epidermal growth factor (EGF).[120] Some mAbs can mediate antitumor activity independent of effector cells, such as by blocking essential survival signals or inducing apoptotic signals. For example, two mAbs approved for clinical use, reactive with the Her-2/Neu receptor on breast cancer cells and the epidermal growth factor receptor on epithelial tumors, provide therapeutic benefits in part by blocking growth signals. The antitumor activity of mAbs can also be enhanced by attaching radioisotopes or drugs or by engineering recombinant bi-specific antibodies that simultaneously bind tumor cells and activate receptors on immune effector cells such as CD3 or FcR.[121]

The efficacy of stimulating a patient's own tumor-reactive B-cells may be limited by the magnitude of the antibody response that can be achieved *in vivo*. Nevertheless, this approach remains appealing because of demonstrations with tumor cell expression libraries that sera from a large fraction of patients already contain tumor-reactive antibodies. The simplest means to stimulate such B-cells *in vivo* is to provide tumor antigens in immunogenic vaccine formulations, such as mixed with adjuvants or conjugated to antigens that can elicit helper T-cell responses. Marked clinical results have been observed after priming patients with autologous dendritic cells (discussed previously). These cells were pulsed with the unique idiotypic immunoglobulin derived from the B-cell receptor of a patient's own B-cell lymphoma followed by boosting with the immunoglobulin conjugated to the helper protein keyhole limpet hemocyanin (KLH).

Alternative approaches for activating and expanding existing B-cell responses *in vivo* by ligation of co-stimulatory molecules, such as CD40 or by administration of the B-cell proliferative cytokine IL-4 have not met with much success in preclinical models and could potentially induce hazardous autoreactive antibodies. Thus, humoral therapy will likely continue to be dominated by passive administration of mAbs specific for selected tumor antigens.

Conclusion

Immunotherapy may be the next great hope for cancer treatment. While monoclonal antibodies, cytokines, and vaccines have individually shown some promise, it is likely that the best strategy to combat cancer will be to attack on all fronts. Clearly, different strategies demonstrate benefit in different patient populations. It may be that the best results are obtained with vaccines in combination with a variety of antigens, or vaccine and antibody combinations. A nonspecific and specific immunotherapy combination offers another potent strategy.

Table 1. Monoclonal antibodies, cytokines and short peptides used in cancer immunotherapy.

Type	Application	Target
Alemtuzumab	Chronic lymphocytic leukemia	CD52
Bevacizumab	Anti-angiogenic therapy	Vascular endothelial growth factor (VEGF)
Cetuximab	Colorectal, head and neck cancer	Epidermal growth factor receptor (EGFR)
Gemtuzumab	Acute myeloid leukaemia	Myeloid cell surface antigen CD33 on leukemia cells
Ibritumomab	Non-Hodgkin's lymphoma	CD20
Nimotuzumab	Squamous cell carcinoma, glioma	EGFR inhibitor
Panitumumab	Colorectal cancer	EFGR
Rituximab	Non-Hodgkin's lymphoma	CD20 on B lymphocytes
Tositumomab	Non-Hodgkin's lymphoma	CD20
Trastuzumab	Breast cancer	HER2/neu receptor
Cytokines		
Interferon-gamma	Melanoma, renal and kidney cancer, follicular lymphoma, hairy cell leukemia	IFN-stimulated gene factor 3 (ISGF3)
Interlukin-2	Melanoma, renal and kidney carcinoma, hematological malignancies	Suppressors of cytokine signaling (SOCS) 1, SOCS2, dual-specificity phosphatase (DUSP) 5, DUSP6
Short peptides		
MART-1, gp100, tyrosine, MAGE-3	Melanoma	
PAP/GM-CSF	Prostate carcinoma	
MAGE-3.A24	Bladder cancer	
Follicular B lymphoma	Idiotype/KLH conjugate	

The effect of any of the aforementioned strategies in combination with more traditional cancer therapies is another promising avenue. Using these concerted efforts, the ultimate achievable goal may be a durable anti-tumor immune response that can be maintained over the course of a patient's lifespan.

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Disclosure

This manuscript has been read and approved by the author. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The author and peer reviewers of this paper report no conflicts of interest. The author confirms that they have permission to reproduce any copyrighted material.

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