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Review Article

VACCINES AND ANTIBODIES FOR CANCER IMMUNOTHERAPY A REVIEW

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ABSTRACT

A better understanding of the molecular and cellular mechanisms controlling the immune system in the past two decades has opened the door to many innovative and promising new cancer therapies that manipulate the immune response. For instance, wide arrays of antigen specific cancer vaccines and cell-based immunotherapies utilizing T-cells, natural killer cells and dendritic cells have been established. Furthermore, a rapidly expanding repertoire of monoclonal antibodies and bispecific antibodies is being developed to treat tumors and many of the available antibodies have demonstrated impressive clinical responses. This review briefly summarizes some of these active immunotherapy (vaccination) and passive immunotherapy (antibody-based therapy) currently in use or under clinical investigations that may soon become part of the therapeutic arsenal to combat cancer in a more efficient way.

Keywords: Cancer, Cancer vaccines, Cancer immunotherapy, Monoclonal antibodies, Bispecific antibodies.

INTRODUCTION

Cancer is a complex disease, with many subtypes and affecting various tissues in diverse ways.[1] It is a spectrum of genetic diseases resulting from various mutations in specific genes. These mutations, which may abrogate gene function or increase gene function, drive cells toward the unregulated and irreversible cellular proliferation that is the hallmark of cancer.[2] Thus, to gain the initiative in cancer detection and treatment, researchers and oncologists must begin to understand the molecular roots of the disease: genes, their messenger RNAs and the proteins they produce.[3] The application of current treatment techniques including surgery, radiation therapy, chemotherapy; result in the cure of more than 50 % of patients diagnosed with cancer.[4] Although such conventional therapeutic approaches may result initially in significant improvement and response, the response duration is brief and in most patients, disease progressions are inevitable and advanced cancers are refractory to conventional therapies.[5] Chemotherapy for the treatment of tumors is often limited by a narrow therapeutic index, bitter side effects of most drugs and emergence of anticancer drug resistance.[6] Moreover, most chemotherapeutic agents do not preferentially accumulate at the tumor site and may need the active targeting of

tumors with particulate drug carriers to enhance the efficacy of the drugs.[6, 7]

Since there is no curative treatment available for most advanced cancer diseases currently, it is important to develop new therapeutic approaches to improve the overall cure rates for advanced cancers. Some of the novel therapeutic approaches under current development include hormonal/endocrine therapy, [8] gene therapy, [9,10,11] thermotherapy,[12] photodynamic therapy, [13,14] antisense therapy[15,16] and immunotherapy.[12,17] Among these approaches, immunotherapy holds great promise because of its capacity to induce a specific immune response existing of a complex of integrated actions of a variety of immune cells, endothelial cells, a wide range of cytokines, growth factors and antibodies directed against tumor antigens to help fight off residual tumor cells and thereby improve survival and quality of life of cancer patients.[18]

Cancer immunotherapy is a major branch of biological therapy that aims to manipulate the immune system to create a hostile environment for cancer cells in the body.[12] It is a treatment modality that mediates tumor lysis through the action of host immune system, based on the assumption that tumor cells express unique proteins, known as tumor antigens, which can be recognized as foreign or non-self by the host resulting in tumor rejection.[17] The treatment stimulates the immune system, utilizing agents that may result in active and /or passive immunotherapy. The active immunotherapy involves vaccination of patients with agents that elicit activation of tumor-specific T-cells. Early efforts in cancer vaccine studies involved the use of irradiated tumor specimens or tumor cells derived from the patient (autologous), or from other individuals (allogeneic) to inoculate cancer patients in hopes of generating a therapeutic immune response.[19]

Until recent years, vaccine design relied exclusively on infectious-attenuated or inactivated-whole viral particles or bacteria or whole tumor cells to establish prophylactic immunity to human pathogens or tumor cells. However, some of these vaccines have adverse side effects. This ironic outcome of the success of vaccination has brought an increased emphasis on safety to the governmental regulatory agencies that must approve new vaccines, as well as to the pharmaceutical concerns producing them. The development of subunit vaccines greatly increases the safety of prophylactic immunization, but also reinforces the need for a new generation of delivery vehicles as well as immunostimulatory adjuvants.[20] In many cases, the subunit vaccines are only very weakly immunogenic; therefore, an adjuvant is needed to intensify the immune response. Adjuvants can also be included in vaccines to guide the type of immune response generated. This may be especially important when developing vaccines for cancer, human immunodeficiency virus or mucosal immunizations. This component may facilitate targeting and/or controlled release of the antigen to antigen presenting cells. Adjuvants and delivery vehicles have also been shown to protect antigens from degradation, although this generally depends on the nature of adjuvant.[21]
Over the past 20 years, the identification of the critical role that dendritic cells (DCs) play in stimulating a specific immune response has led to their use in cancer immunotherapy.[22] DCs are the professional antigen presenting cells of the immune system that possess the unique capacity to take up and process antigen, migrate to the draining lymph nodes and present antigen to resting T-lymphocytes**.** DC- based vaccines have been used to stimulate immune responses, in particular those responsible for combating cancer.[23]The passive immunotherapy is the other important wing for cancer immunotherapy that involves administration of activated immune system effector component into cancer patients. This strategy includes particularly the use of cancer specific monoclonal antibodies (MAbs) and bispecific antibodies (BsAbs) to stimulate the effector mechanisms against cancer cells and

to target the "payloads" such as toxins, radioisotopes, enzymes or cytotoxic drugs in to cancer cells.[19]

IMMUNITY TO TUMORS

Cancer immunotherapy aims at eliciting an immune response directed against tumor antigens to help fight off residual tumor cells and thereby improve survival and quality of life of cancer patients.[24] The major effector mechanism for the immune systems to attack tumor cells is generally considered to be cell mediated cytotoxicity. Macrophages, T- lymphocytes and natural killer (NK) cells are generally considered as potential cytotoxic effector cells in the anti-tumor immune response.[25] DCs that are pulsed with tumor antigens are also important players in the immunotherapy of cancer. They are highly specialized in antigen capturing, processing and presentation and express co-stimulation signals which activate T- lymphocytes and NK cells against cancer**.** The functions of these cells in response to cancer are schematized in Figure 1. Furthermore, a rapidly expanding repertoire of monoclonal antibodies is being developed to treat tumors, and many of the available antibodies have demonstrated impressive clinical responses**.**[26, 27]

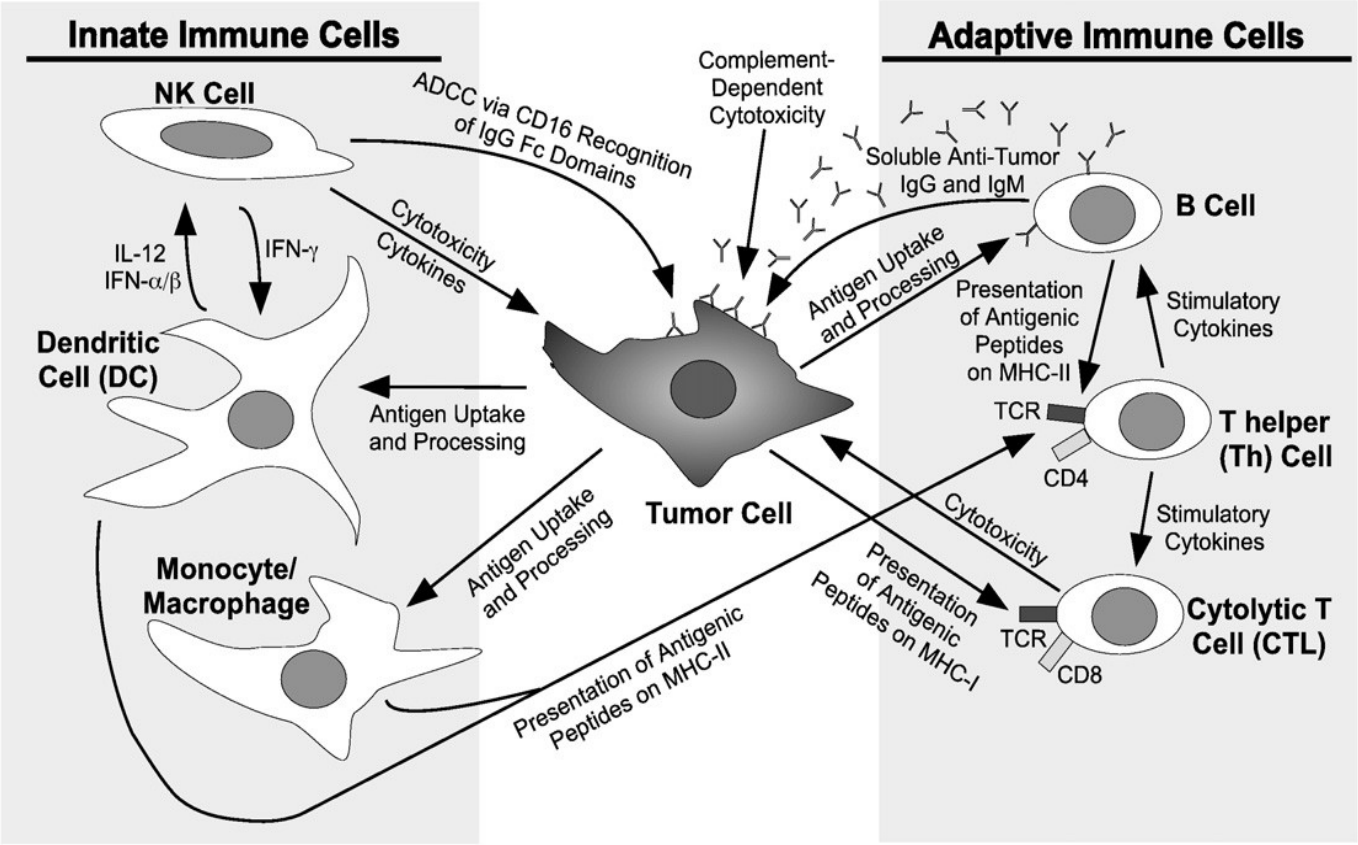

Figure 1: Major cells of the innate and adaptive immune systems and their functions in response to a tumor cell. Grey boxes delineate the major cell types constituting the innate and adaptive immune systems. Arrows describe impacts of immune cells on each other or the tumor.
Note that this is a very basic schematic designed to define interactions of relevance to the review, and numerous complex molecular interactions and minor immune cell subsets further influence these functions.[27]

Role of T- Cells In Antitumor Immune Response

T- Lymphocytes are the key effector cells of the adaptive immune response. They are developed from a common lymphoid progenitor in the bone

marrow that also gives raise to B- lymphocytes but those cells destined to give rise to T- cells leave the bone marrow and migrate to the thymus for development into T-helper cells or T-killer cells.[28] Traditionally, T-killer cells have been considered the only component necessary for the elimination of malignant tissue, while T helper cells were thought of as mere providers of additional stimuli. Indeed, T-killer cells can fail to maintain functionality *in vivo* in large part because of the absence of T-helper cells help.[29] T-helper cells, upon activation by antigen presenting cells, differentiate into cytokine-expressing effector cells, which are classified as Th1, Th2, Th17 and T follicular helper (Tfh) cell subsets on the basis of their cytokine secretion and immune regulatory function.[30] T-helper cells play a central role in virtually every aspect of immunity, including the antitumor response. The addition of antigen-specific helper cells to T-killer effector cells could increase the rate of clinical success of adaptive immunotherapies. T-helper cells can be also directly cytotoxic to major histocompatibility complex (MHC) class II-expressing target cells**.** They also aid the B-cells to make antigen specific antibodies.[29, 31]

Role of Natural Killer Cells and Macrophages

NK cells are commonly defined as the effectors of the innate immune system, acting as the first line of defense against viral infection and tumors. They do these through direct killing of virally infected cells or lysis of transformed cells and through production of cytokines that are crucial for both controlling infection and immune surveillance.[32] Cell-surface NK receptors recognizing MHC class I and class I-like molecules signal for inhibitory and activating NK cell function, thus enabling NK cells to 'recognize' and selectively lyses foreign or transformed or infected cells.[33] NK cells produce a wide array of cytokines, including IFN-γ, tumor necrosis factor alpha (TNF-α), granulocyte macrophage colony stimulating factor , macrophage colony stimulating factor , Interleukin-2 (IL-2), IL-3, IL-5 and IL-8. This cytokine profile of activated NK cells skews the helper T lymphocyte response and activates macrophages and thus influences the development of the adaptive immune response. NK cells have also been shown to induce antibody production by B-cells and even function as antigen presenting cells to specific T-cell clones in a MHC Class II restricted manner. Moreover, absence of NK cells prevents the induction of cytotoxic T- lymphocytes. Thus, NK cells appear to play an important role in modulation of B- as well as in T-lymphocyte mediated immunity.[34] Macrophages are found in many areas of lymph node, especially in the marginal sinus, where the afferent lymph node enters the lymphoid tissue and in medullar cords, where the efferent lymph collects before flowing in to the blood. These cells play a pivotal role in host defense against cancers. The major functions exerted by these cells include cell mediated and antibody dependent cytotoxicity and phagocytosis.[35]

Destruction of tumor cells by macrophages requires activation of macrophages and cell to cell contact. Activated macrophages are able to distinguish normal cells from tumor cells and kill only the latter by release of cytotoxic compounds such as IL-l, TNFα, superoxide anion, hydrogen peroxide, nitric oxide and proteolytic enzymes. Since activated macrophages kill tumor cells which are phenotypically different and particularly resistant, especially to anticancer drugs and host defense, the choice of these cells as effectors in immunotherapy targeting biologically heterogeneous metastatic cells could be highly advantageous. The ability of macrophages to phagocytose turnout cells further allows them to act as antigen presenting cells for cytotoxic T-cells.[36]

Role of B Lymphocytes

B Lymphocytes control humoral immunity and represent the effector cell type involved in the production of antibodies. The generation of antibody-secreting plasmacytes and memory B cells occurs in secondary lymphoid organs. An immune response is initiated when specialized

antigen presenting cells such as DCs acquire antigen in the periphery and migrate to the draining lymph node where the antigen comes into contact with naïve or memory lymphocytes. Antigen-specific B cells presented with their complementary antigen become activated and undergo developmental events in the germinal centre, including somatic mutation of immunoglobulin V genes and affinity selection, which lead to a large clonal expansion of antigen-specific plasma cells and memory B cells. [36, 37] MAbs, which are secreted by these lymphocytes, bound to tumor antigens to induce tumor cell death. There are several mechanisms through which antibody-mediated mechanisms are likely to be important in controlling and eliminating tumors. The main mechanism is called antibody dependent cell-mediated cytotoxicity, in which antibodies bound to tumor cells activate effector cells of the immune system against tumor cells. For instance, receptors expressed on NK cells and other leukocytes bind to the antibody-tumor cell complex through the antibody Fc region, release cytotoxic granules containing perforin and granzymes and destroy the tumor cells.[38]

Role of Dendritic Cells

The dendritic branch which is made up of cells derived from the bone marrow assists T- cells. For T- cell to respond to antigen, the antigen must be processed and presented by DCs to the T-cell resulting in T-cell activation.[31] DCs are potent antigen presenting cells that possess the ability to stimulate naïve T- cells. They comprise a system of leukocytes widely distributed in all tissues, especially in those that provide an environmental interface. DCs posses a heterogeneous haemopoietic lineage, in that subsets from different tissues have been shown to possess a differential morphology, phenotype and function. The ability to stimulate naïve T-cell proliferation appears to be shared between these various DC subsets. It has been suggested that the so-called myeloid and lymphoid-derived subsets of DCs perform specific stimulatory or tolerogenic function, respectively.[39]

CANCER VACCINES

Immunotherapy may be classified into several types, including, (1) active immunotherapy-specific stimulation of patient's immune system with vaccines, and/or nonspecific stimulation using adjuvants; (2) passive immunotherapy treatment with exogenously produced antibodies; (3) adoptive immunotherapy-transfer of lymphocytes and/or cytokines; (4) restorative-designed to restore deficiencies in the patient's immune response; and (5) cytomodulatory meant to enhance the expression of MHC molecules on the surface of the tumor cells.[40] The use of cancer vaccines, which exemplify active specific immunotherapy, usually combined with adjuvants, is one of the rational strategies for the treatment of cancer. Successful cancer vaccination to induce immunity against tumor antigens could lead to tumor cell destruction and prolong the survival of cancer patients. A variety of strategies have been used to enhance the antigenicity of the tumor cells, including genetically modifying the cells to secrete cytokines involved in antitumor immunity and initiating a viral infection for the "xenogenization" of the tumor cells. A major advantage of these methods is that identification of the tumor antigens is not required, and theoretically, immunization with multiple tumor antigens, including tumor antigens specific for individual tumors, is possible.[41]

Vaccines That Activate T Cells

One approach to inducing tumor specific immune responses is to genetically modify tumor cells to express MHC molecules and costimulatory molecules on their surface that are critical for T-cell activation. Modification of the vaccinating tumor cell in this way enhances the tumor's ability to directly stimulate T-cells. The modification of tumor cells to function as professional antigen presenting cells that directly activate T-cells can be accomplished by both *in vitro* and *in vivo* gene transfer strategies. This is also true for cancer vaccine approaches that

manipulate professional antigen presenting cells to enhance tumor specific T-cell activation. [42, 43]

Vaccines That Attract Antigen Presenting Cells

Studies have indicated that professional antigen presenting cells in the periphery, such as DCs and macrophages, play an important role in initiating tumor specific immune responses.[44] This can be achieved by targeting the immunizing antigen to professional antigen presenting cells, which can process and present the antigenic peptide to the T-cell in the presence of constitutively expressed costimulatory molecules. Depending on the type of vaccine, targeting of antigens to antigen presenting cells can be accomplished through a number of different pathways. One central pathway for targeting of vaccinating antigens into bone marrow– derived antigen presenting cells is the exogenous pathway of antigen uptake. The success of this pathway, operative in most cell-based as well as protein-based vaccines, depends largely upon the adjuvant with which the tumor cell or tumor antigen(s) is formulated. The adjuvant is a critical component of a vaccine that attracts antigen presenting cells to the sight of vaccination, where they then become activated and take up antigen for processing and presentation. Another pathway for introduction of antigens into appropriate bone marrow–derived antigen presenting cells is via direct transduction. This can be achieved by certain recombinant bacterial and viral vaccines as well as nucleic acid vaccines, so-called ‘‘naked DNA’’ vaccines. In addition, antigenic peptides can be loaded directly on to empty MHC molecules on the surface of antigen presenting cells either *ex vivo* or *in vivo*, thereby bypassing the processing steps.[43]

Specific Vaccine Strategies

Cell based cancer vaccines

Until the tumor antigens expressed by most tumors are identified, the tumor cell itself will continue to be the best source of immunizing antigens. This vaccination strategy involves a whole cell vaccine using autologous or allogeneic fresh tumor cells or tumor cell lines.[43,45] Autologous tumor cell vaccines, in which tumor cells are extracted from surgical resection or biopsy specimens, attempt in a controlled fashion to immunize the patient against all the antigens that are endogenously expressed by their tumor.[46] The use of autologous tumor cells to make individualized vaccines is certainly optimal; however, it does have its drawbacks. To accommodate multiple or even a single vaccination requires a reasonable amount of fresh tumor biopsy.[45] Allogeneic cell lines have also been developed for tumors such as melanoma that likely encompass many of the tumor associated antigens expressed by the melanomas of most affected individuals.[46]

Tumor cells can also be modified to make them more immunogenic. To accomplish that, tumor cells may be infected with various types of viruses so that viral proteins are expressed on the surface and transduced with genes expressing cytokines such as IL-2 and granulocyte macrophage colony stimulating factor or co-stimulatory molecules. The modified cells may be irradiated to prevent their proliferation and then injected back in to the patient. With the development of improved genetic techniques, the concept of presenting immunologically defined ‘‘adjuvants’’ at the same site as tumor antigens in order to augment antitumor immunity has been also tested. The immune system can be activated by either the tumor cell or the inflammatory response that includes recruitment of DCs. As the injected tumor cells undergo apoptosis or are destroyed by the inflammatory reaction, antigens are picked up by the DCs and represented to the T-cells.[45, 46]

Antigen specific cancer vaccines

Plasmid DNA based vaccines

Tumor cells over express specific antigens which allow them to be recognized and destroyed by the immune system. Triggering antitumor immunity in cancer patients by specific vaccination is foreseen as a safe and versatile

method to control cancer.[47] There is now a range of potential target antigens including cell surface molecules, susceptible to antibody attack and a multitude of intracellular antigens, requiring cytotoxic T-cells.[48] Whole tumor cells, nucleic acids, proteins or derived peptides have been used as a source of antigen.[47]

The advent of genetic technology allows construction of DNA based vaccines encoding selected tumor antigens together with molecules to direct and amplify the desired effector pathways.[48] Plasmid DNA based cancer vaccines have many inherent features that make them promising cancer vaccine candidates. Although plasmid DNA vaccines and recombinant viral vectors can induce antibody and T-helper cell responses, they are particularly suited to induce cytotoxic T-cell responses because they express antigens intracellularly, introducing them directly into the MHC class I antigen processing and presentation pathway.[49] Plasmid DNA can express the antigen *in vivo* after intradermal, intramuscular, intravenous or subcutaneous injection. The expression triggers the development of antibody mediated and cell mediated immune response specific for the antigen.[47] The main disadvantage of plasmid DNA vaccines is their poor immunogenicity that might need different strategies such as the use of adjuvants to enhance the immunogenicity of such vaccines.[49]

Recombinant viral and bacterial vaccines

Microorganisms are the cause of 10-20 % of all human tumors. Vaccines that control or prevent virus associated infections that are linked to cancer promotion are an effective and important primary preventive strategy in cancer. To date, two such vaccines are available: one to Hepatitis B virus that reduces the likelihood to develop hepatocellular carcinoma and the other for human papilloma virus (HPV), to protect against cervical cancer. The HPV vaccine is the first vaccine explicitly designed to prevent cancer induced by a virus but the hepatitis B vaccine was not primarily designed to prevent cancer. The finding that infection with HPV is a critical factor in the majority of cases of cervical cancer allowed the development of strategies to prevent this form of oncogenesis.[50]

Several recombinant viral vectors are currently undergoing rigorous testing for their ability to augment antitumor immune responses against model tumor antigens. For example, the use of viruses such as vaccinia virus, small pox viruses, adenoviruses and retroviruses for the transfer of plasmid DNA to a mammalian host cell is a well established and has already made its way to clinics for gene therapeutic interventions or vaccine trials.[43, 51]

Genetic immunization with plasmid DNA vaccines has proven to be a promising tool in conferring protective immunity in various experimental animal models of infectious diseases or tumors. More recently, some bacterial species, in particular enteroinvasive species, were used as effective carriers for DNA vaccines for cancer immunotherapy. Attenuated strains of *Shigella flexneri, Salmonella spp., Yersinia enterocolitica* or *Listeria monocytogenes* have shown to be attractive candidates to target DNA vaccines to immunological inductive sites at mucosal surfaces.[51]

Peptide or protein based vaccines

Protein and peptide based vaccine strategies involve the administration of high doses of proteins/peptides that can be loaded onto empty MHC molecules on antigen presenting cells *in vivo*. One major advantage of protein/peptide vaccination is that it removes the safety concerns associated with using live recombinant vaccines or DNA vectors. Whereas vaccination with some tumor associated peptides and proteins have induced systemic antitumor immunity, the administration of other peptides has led to the induction of tolerance rather than activation. Tolerance may be directed toward immune dominant epitopes of self-proteins, which are toleragenic. In animal model systems, tolerance to self-proteins can be circumvented by targeting

the immune response to non-immunodominant peptide portions of the self-tumor antigen, i.e., a subdominant epitope. However, there are no standard regimens for immunizing humans to peptide portions of self-tumor antigens.[52]

Antigen pulsed dendritic cell vaccines

DCs are bone marrow derived cells that function as the most potent professional antigen presenting cells of the immune system for their unique capability of presenting antigen to T-cells.[53] They are a heterogeneous population of antigen presenting cells identified in various tissues, including the skin, lymph nodes, spleen and thymus.[54] DCs are considered to be initiators and modulators of immune responses and are capable of processing antigens through both MHC class I and II pathways.[55] The antigens that are displayed on the membrane of DCs along with MHC class II and MHC class I proteins can be recognized by T-helper cells and cytotoxic T-cells respectively through their T-cell receptors. Recognition of the antigens as non-self is important for stimulating T-cells and secretion of cytokines that induce T-cell differentiation that is critical for induction of T-cell responses to pathogens and tumors.[56]

The ability of a vaccine to prime the immune system for an effective response upon antigen exposure is dependent on the antigen specific activation of effector T- and B- cells of the adaptive immune system. Due to their ability to activate naive T-cells, DCs play a key role in initiating an immune defense against infectious diseases and cancer.[57] Therefore, a novel approach to vaccination against cancer is to exploit DCs as 'nature's adjuvants' and actively immunize cancer patients with a sample of their own DCs primed with tumor antigens.[58] DC may represent the key to the development of novel vaccination approaches that mimic the course of natural immune responses or trigger de novo responses that have been ignored or suppressed.[59] In the past few years, several *in vitro* and *in vivo* studies in rodents and humans have demonstrated that immunizations with DCs pulsed with tumor antigens result in protective immunity and rejection of established tumors in various malignancies.[60] These studies have evaluated a range of tumor antigens as well as used varying formulations such as defined MHC class I peptide epitopes, full-length recombinant proteins, specific or total tumor cell mRNA, virally delivered DNA, autologous tumor cell lysates or whole tumor cells and even allogenic tumor cell lines.[61] Since the use of live vaccines can have safety concerns, current strategies often focus on the use of subunit vaccines such as protein or peptide antigens and DNA vaccines. However, this approach can result in the production of vaccines that have poor immunogenicity and efficacy, or result in the induction of an inappropriate response such as tolerance rather than immunity.[57] To optimize DC vaccination, it will be important to design strategies for appropriate activation of DC, improved antigen delivery to DC and in situ targeting of DC.[59]

Results from various vaccination studies, in animal models and in humans, stress the need for different vaccine delivery systems that are required to introduce non-replicating antigens into the MHC class I and II presentation pathway and trigger the expression of co-stimulatory molecules on DCs. These strategies increase vaccination efficacy as well as improve control on the immunological outcome.[62] An immunization strategy based on the use of different adjuvants such as liposomes,[57] virosomes,[62] virus like particles,[63] poly (dl-lactide- *co*-glycolide) microspheres,[64, 65] cell penetrating peptides,[66] monophosphoryl lipid A [67] and immune stimulating complexes [68] can achieve these goals. Different studies had shown that co-delivery of subunit vaccines with adjuvants that possess the ability to induce DC maturation to DCs have resulted in effective DC-based vaccines for immunotherapy of cancers.[63] Moreover, most of these adjuvants generally offer the ability to protect antigen from degradation and deliver specifically to DCs and release the antigen intracellularly so that it can

be processed by both MHC class I and class II pathways to induce DC maturation and to initiate adaptive immune responses, especially initiation of a CD8+ T-cell response, for immunotherapy of cancer and viral infections.[65, 69]

ANTIBODIES FOR CANCER IMMUNOTHERAPY

Antibodies are large serum proteins that are produced by B-lymphocytes and bound very tightly to their target antigens. They are usually produced in response to their specific antigens from microbes, cancer cells, transplants or any exogenous proteins. Antibody preparations have been used for several decades to induce passive immunization against such a wide range of antigens. The ability of antibodies to bind an antigen with a high degree of affinity and specificity has led to their ubiquitous use in a variety of scientific and medical disciplines.[70, 71] They have become common and essential tools in western blotting, immunohistochemistry, immunocytochemistry, enzyme linked immunosorbent assay, immunoprecipitation and flow cytometric analysis.[72]

Passive immunization that is induced by antibody preparations can be used prophylactically, to prevent a future medical episode, or therapeutically to treat a medical condition that is already established. Antibody based therapy has emerged as an integral part of effective treatments for autoimmune diseases, transplant rejections, respiratory diseases, cardiac diseases, infectious diseases and cancers.[72-75] Paul Ehrlich, a century ago, described the antibody therapy concept to selectively target malignant cells based on the unique expressed determinants profile of the disease (the "magic bullet" hypothesis). It is effective through a variety of mechanisms. It can block essential cellular growth factors or receptors, directly induce apoptosis, bind to target cells and recruit 'effector functions' such as antibody dependent cellular cytotoxicity or complement dependent cytotoxicity and deliver cytotoxic payloads such as radioisotopes and toxins to the target cells.[76,77]

The transformation of a cell to the cancerous state is normally associated with increased surface expression of antigens recognized as foreign by the host immune system. However, the immune response elicited by tumor antigens are not inherently strong because they are recognized as self cells given that tumor antigens are self antigens.[78] Thus to strengthen the host immune response, highly specific immunotherapeutic agents should be used to increase the immunogenicity of the tumor cells. In the past three decades, highly specific MAbs have been designed to target and destroy cancer cells by recognizing specific antigens on their surface.[79, 80] More recently successful MAb-based therapies were targeted to molecules involved in the regulation of growth of cancer cells. These antibodies were also used for enhancing the cytolytic mechanisms against the tumor cells. MAb- based medicines harbor tremendous potential for cancer immunotherapy and a number of such products have gained approval for clinical purposes.[81]

Monoclonal Antibodies in Cancer Therapy

MAbs were first described by Köhler and Milstein in 1975. The discovery, which was reported in *Nature* and eventually, earned the two scientists a Nobel Prize in 1980, generated great excitement and expectations in the field of immunology.[80] These are antibodies which have a single, selected specificity and which are continuously secreted by immortalized hybridoma cells that are a biologically constructed hybrid of a mortal, antibody-producing, lymphoid cells and malignant or immortal myeloma cells.[82] Great strides were made in the analysis of a variety of cell types and the focus of attention among researchers, clinicians and especially biotechnology companies shifted to the diagnostic and therapeutic potential of the new reagents. At the crux of interest in therapeutic MAbs was their promise for use in the treatment of cancer. The

original design was twofold: the first step was to identify tumor specific antigens by means of mAb and the second to trigger a lethal attack against tumor cells without the involvement of normal cells lacking the tumor marker.[81]

In the past three decades a total of 200 unique therapeutic MAbs where studied in clinical trials by different groups worldwide for various cancer indications. To date, some of these anti-cancer MAbs have been approved for use indifferent clinical settings for different types of cancers.[31] Some of the most commonly used MAbs for cancer immunotherapy are shown in Table1.

Table 1: Some of the monoclonal antibodies thus far approved for cancer immunotherapy[10,31,78, 83, 84]

Product	Indication
Arcitumomab	Detection of recurrent/metastatic colorectal cancer
Satumomab Pendetide	Detection/staging/follow up of colorectal and ovarian cancers
Capromab Pentetate	Detection/staging/follow up of prostate adenocarcinoma
Rituximab	Non-Hodgkin's lymphoma
Nofetumomab	Detection of small cell lung cancer
Trastuzumab	Treatment of metastatic breast cancer
Igovomab	Diagnosis of ovarian adenocarcinoma
Votumumab	Detection of carcinoma of the colon or rectum
Alemtuzumab	Chronic lymphocytic leukaemia
Gemtuzumab Zogamicin	Acute myeloid leukaemia
Ibritumomab Tiuxetan	Non-Hodgkin's lymphoma
Tositumomab	Non-Hodgkin's lymphoma
Cetuximab	Treatment of EGF receptor expressing metastatic colorectal cancer
Avastin	carcinoma of the colon or rectum
Bevacizumab	Treatment of colorectal cancer

Most immunotherapeutic approaches using MAbs are based on the concept of targeting tumor associated antigens that are expressed to a greater extent on the surface of tumor cells than on normal cells and tissues.[85] Once the antibody recognized a malignant cell, it can elicit its antitumor effect by suppressing tumor growth and dissemination by using the natural effector mechanisms of antibodies to destroy tumor cells. These include antagonizing receptor tyrosine kinases that are vital for tumor cell proliferation and transformation , directly inducing tumor cell apoptosis and eliciting immunological effects such as antibody dependent cellular cytotoxicity and complement dependent cytotoxicity following activation of the complement cascade in proximity to the tumor cells, with the formation of the membrane attack complex consisting of the complement components C5-C9 and the generation of chemotactic fragments, for example, C3a and C5a. The latter have the ability to attract phagocytic cells, such as monocytes, macrophages or NK cells, which can use their Fc receptors to lyse tumor cells mediated by antibody dependent cellular cytotoxicity.[80, 85-87] MAb-based therapeutic

agents can also achieve their therapeutic effects by inducing tumor regression via the anti-idiotype network. Specifically, immune-competent hosts are vaccinated with an anti-idiotypic antibody mimicking the antigenic determinant of the original immunogen, which ideally is recognized by B-cells and followed by a humoral response, leading to the endogenous production of tumor-specific anti-idiotypic antibodies.[85, 87] In addition, MAbs serve as vehicles to deliver cytotoxic substances, such as radionuclides, chemotherapeutic drugs or toxins to the tumor cells in order to elicit a tumor specific cytotoxic effect.[78] This strategy increases effectiveness and reduces non specific toxicities of radio-isotopes, toxins, drugs or enzymes because the antibodies can selectively bind with antigen bearing cells and deliver their "payloads" directly to tumor cells.[88] Radiolabeled MAbs directed against tumor associated antigens or tumor surface antigens selectively concentrate the radiolabel at the site of the tumor, allowing imaging of the primary tumor and/or metastases or site selective delivery of radio-isotopes for therapeutic use. Immunotoxins are composed of an antibody targeting moiety and a toxin moiety for cytotoxicity. Natural ribosome-inhibiting protein toxins from plants, bacteria and fungi can be either chemically attached to the antibody fragment or via fusion at the molecular level. Theoretically, one molecule of toxin delivered intracellularly at the appropriate compartment will be lethal to the tumor cell. Conjugation of antineoplastic agents such as daunomycin or methotrexate to antibodies resulted in a marked decrease in cytotoxicity of the drug and increase in selectivity of target cell killing.[87] Several of these approaches resulted in some successes in clinical applications when applied to certain sensitive tumors, such as non-Hodgkin lymphoma. But most naked MAbs when used as mono-therapy are only modestly effective against solid tumors. The one exception to this is trastuzumab, which targets the HER-2 growth factor receptor and is used in the treatment of metastatic breast cancer.[76, 89]

In the so called antibody directed enzyme prodrug therapy approach, an enzyme is coupled to a target cell directed antibody. After administration, the enzyme-MAb complex is allowed to bind to its target. Subsequently, a non-toxic, prodrug is administered. Upon exposure to the enzyme-MAb complex, the active drug is enzymatically cleaved from the prodrug and released locally at high concentrations. This strategy is one of the various tumor targeting approaches to improve the efficacy of anticancer chemotherapy by reducing the adverse side effects and damage to normal tissues associated with systemic dug delivery and therapy.[87, 90-92] This therapeutic approach seems to be justified in an adjuvant setting for the treatment of minimal residual disease or leukaemia or after surgery of the primary tumor to kill possible circulating tumor cells. However, by using this approach few clinical responses were observed in the treatment of solid tumors due to heterogeneous and low uptake of conjugates of anticancer agents and antibody or pro-antibody fragments. To overcome the problem of delivery of MAbs for the therapy of larger tumor masses, smaller fragments such as single chain variable domain fragments (ScFv) and more easy accessible target cells such as tumor vascular endothelial cells have been studied.[87,93] Moreover, it is clear that application of the advanced and new molecular technologies to refine the macromolecular structure of the MAbs to maximize tumor targeting and penetration will be of great utility in improving the efficacy of antibody based cancer immunotherapy.[80, 89]

Bispecific Antibodies in Cancer Therapy

Using hybridoma fusion, chemical derivatization or molecular biology technology, antibodies with dual specificity can be constructed. These hybrid MAbs, so called bispecific antibodies (BsAb), elicited possibilities to combine tumor cell and immune effector cell specificicities in a single

antibody molecule.[87] BsAbs have been used to redirect the cytolytic activity of a variety of immune effector cells such as cytotoxic T-lymphocytes, NK cells, neutrophils and monocytes/macrophages to tumor cells.[94]

For use in cancer immunotherapy, most immunotherapeutic strategies exploiting the application of BsAbs with dual specificities, one for tumor specific antigens and another for effector cells as shown in Figure 2. Upon proper activation of the effector cells, the BsAb enables them to redirect their cytolytic activity towards the tumor cells.[87] BsAbs redirect and trigger cytotoxic T- lymphocytes to mediate tumor cell lysis regardless of their initial antigen specificity. In addition, the interaction between redirected cytotoxic T-lymphocytes and tumor cells is independent of MHC antigens, so that the cytotoxicity is not affected by MHC alteration or down regulation on the tumor targets, by which cancers effectively evade the immune attack.[87,95] Various investigators have demonstrated that BsAbs can enhance specific cytotoxicity and exert strong effects *in vivo* when administered either alone or in combination with autologous effector cells. For example; studies in mouse models have shown that BsAbs could eradicate or slow down the tumor growth. Human clinical trials using BsAbs in cancer patients have also shown promising results.[94, 96]

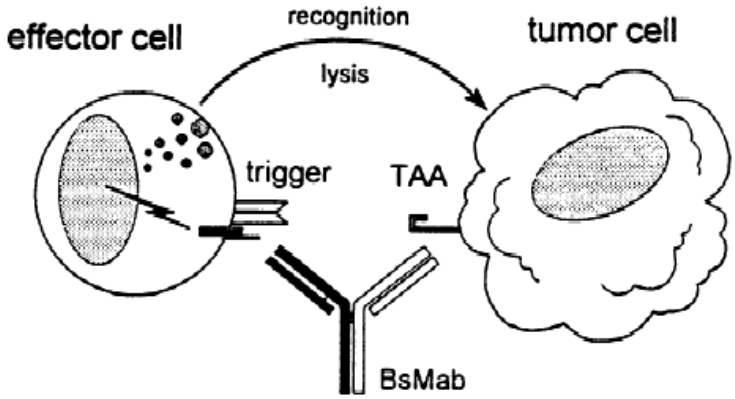

Figure 2: BsMAb mediated tumor cell recognition and lysis by an immune effector cell.[87]

In addition to their use in redirecting effector cells to the tumor site, BsAbs have been also used for targeted delivery of a "payload" such as a toxin, a radioactive hapten or a cytotoxic agent to the tumor site.[97, 98] The role of BsAbs for targeting tumor antigens to DCs to induce differentiation of tumor specific cytotoxic T-lymphocytes have been investigated.[99] BsAbs have been also exploited in a large variety of applied technologies such as immunohistochemistry, enzyme immunoassays and for studying cell–cell interactions.[87]

FUTURE PROSPECTIVE FOR VACCINE AND ANTIBODY BASED CANCER IMMUNOTHERAPY

Now there are some immunological products available commercially in market for the immunotherapy of different cancers. Although manipulating the immune system using vaccines and antibodies to mediate tumor regression is well demonstrated in experimental and clinical settings, impediment remains when translating this into large clinical application. The main obstacles in cancer immunotherapy remained limited accessibility of both the cellular and humoral immune effector agents to the target tumor sites in particular inside solid tumors and the difficulty to obtain a sufficient number of cytotoxic T-lymphocytes and potent specific antibodies. Thus, still different approaches should be explored to circumvent these obstacles and to render immunological interventions more efficient. Especially, efforts should be focused more on gene transfer and genetic engineering to modify effector cells, to design highly specific

antibodies and antibody-conjugates. The development of appropriate adjuvants and delivery systems for safe and effective subunit cancer vaccines is one of the potential approaches that have to be further explored in the future in order to augment active immunization against a wide range of cancers. Moreover, immunotherapy will likely not be able to eliminate tumors alone, but combination therapies that incorporate vaccines, antibodies and other immunotherapeutic agents have great potential for providing clinical success in treating cancer in the coming years.

SUMMARY

Vaccines and antibodies are immunological products that are used to induce active and passive immunity respectively in a living host against infectious agents and tumor antigens. Their applications for the immunotherapy of different cancers gained more popularity in the last two decades. MAbs, BsAbs and different cancer vaccine types have now obtained an entrance ticket for the hospital, but further maturation is required. Failure rates of conventional cancer treatments based on the use of surgery, radiotherapy or chemotherapy are dramatic enough to encourage basic and clinical researchers to meet effective manipulation of the immune system using targeted vaccine and/or antibody based therapies that offer new possibilities to harness the immune response to treat cancer patients.

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