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Minireview: Immunotherapy and its role in cancer

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Abstract

Immunotherapy entails treatments that stimulate, enhance or inhibit a patient's own immune system to challenge a specific disease. The immune system is capable of distinguishing between healthy and tumorigenic cells and the following treatments are aimed at those cells that become cancerous. There are three approaches employed in immunotherapy namely monoclonal antibody administration, immunotherapy making use of cytokines and vaccine-based immunotherapy. Artificially produced monoclonal antibodies are employed to target tumorigenic cells by exerting an antagonistic effect on growth factor receptors or to contribute to the induction of antibody-dependent cell-mediated cytotoxicity. These actions result in the disruption of tumorigenic cells or improvement of the immune response directed against tumorigenic cells. Monoclonal antibodies blocking cytotoxic T-lymphocyte associated protein 4 resulted in tumor regression. Several cytokines are responsible for the stimulation of immune responses directed against tumorigenic cells. Various cytokines also stimulate tumor necrosis factor family members for induction of apoptosis in tumorigenic cells. Vaccines entail an active immunotherapeutic approach in which an immune response is induced by the external administration of antigens. Three different cancer vaccines are currently in use namely vaccines preventing cancer recurrence of treated cancers, eradication of cancer cells not destroyed by previous treatment and targeting of cancer-causing viruses. However, it is clear that immunotherapy should be used in combination with other known treatments to have the optimal effect.

Key words: immunotherapy, monoclonal antibodies, cytokines, vaccines

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Introduction

A specific disease is combated by immunotherapy by stimulating, improving or inhibiting a patient's immune system [1]. Immunotherapy includes passive and active immunization that are aimed at improvement of the individual's immune response to a foreign- or self-antigen (*e.g.* in the case of autoimmunity) [2].

Dendritic cells, B cells and macrophages are professional antigen presenting cells (APC) [3] that constitutively express class II human leukocyte antigen (HLA) molecules [4]. They act at the interface between the peripheral tissue and lymphoid organs and play a vital role in antigen capturing, processing and antigen presentation that stimulate natural killer (NK) cells and T lymphocyte responses. Maturity of dendritic cells is induced by pathogen-associated molecular patterns (PAMPs), Toll-like receptors (TLRs), inflammatory cytokines and prostaglandins [5]. The viability of dendritic cells is determined by pro-apoptotic and anti-apoptotic B-cell/lymphoma 2 (Bcl-2) family of proteins that are influenced by PAMPs via TLRs [4]. Apoptosis comprises a specific molecular sequence of events that lead to cell death and plays a key role in the regulation and maintenance of cell populations in tissues including those of tumor cells [6]. Dendritic cells can thus be controlled by exposure to cytokines that can either trigger dendritic cells to be more resistant to apoptosis or express anti-apoptotic proteins [4]. Since the role of dendritic cells differs in diverse diseases, immunotherapy treatment will thus vary when dendritic cells are involved [3].

Immunotherapy using monoclonal antibodies pertains to the administration of external antibodies that are directed against a specific target [7]. Since cytokines form an integral part of the immune system, it appears reasonable to employ cytokines by external administration or to inhibit certain cytokines using monoclonal antibodies in immunotherapy [8]. Active immunization concerns vaccines and involve the administration of antigens (*e.g.* tumor associated antigens (TAA's) or autoantigens in various autoimmune diseases) that may induce an antigen-specific tolerance [9].

Most immunologists agree that the immune system plays a role in cancer development and significant potential lies in using the immune system in cancer treatments. The body has the ability to recognise between healthy, transformed and cancer cells [10]. Cancer immunotherapy generally aims at producing an immune response specifically directed at the tumor antigens thereby improving time and quality of life [5] in cancer patients by sparing surrounding tissue [11].

William Coley (1862-1936) mainly contributed to cancer research [10] and surgery [12]. It was observed that infections often followed rare spontaneous tumor regression [13]. Paul Erlich (1909) theorized that cancer cells can be attacked by the body's own immune response [10]. However, the latter could not be put to the test in experimental procedures [10] due to an incomplete understanding of the immune system [13]. Fifty years later, Thomas (1959) and Burnet (1970) suggested that lymphocytes have the ability to survey and destroy newly arising tumorigenic cells that are continuously produced in the body [10]. The latter was defined the cancer immuno-surveillance theory. Research performed in the 1970's appeared to hugely discredit the cancer immuno-surveillance theory. These experiments involved nude mice with an atrophic thymus; thus deficient in T cell production. These mice had no increased prevalence of tumors [10]. However, at a later stage the experiments were found to be lacking in several areas. Nude mice have a normal amount of NK cells and a detectable amount of T cells. NK cells can have a profound effect on tumor cells and T cells together with an intact immune system and can have a considerate effect in controlling the number of spontaneous- and induced tumors in nude mice. New information revalidated the immuno-surveillance theory which re-established the hope in immunotherapy research. Twentieth century immunotherapy research was continued in anticipation of a breakthrough in cancer therapy [10].

Monoclonal antibodies and cancer immunotherapy

Monoclonal antibodies can be artificially produced to specifically target cancer cells or TAA's. When injected into a patient, it is anticipated that antibodies will target cancer cells, since monoclonal antibodies of defined specificity are capable of detecting a single antigenic epitope on cancer cells in a heterogeneous population. This leads to the disruption of cancer cells or enhancement of the immune response directed against cancer cells or its effects [7].

Monoclonal antibodies may act by antagonising the effects of growth factor receptors or by inducing antibody-dependent cell-mediated cytotoxicity. These antibodies bind to target antigens and are recognized via Fc-receptors present on surfaces of NK cells, monocytes and macrophages resulting in antibody-dependent cellular cytotoxicity (ADCC). Complement may also be activated by the monoclonal antibody's Fc region, ultimately culminating in the activation of the cytolytic membrane attack complex (MAC or C5b-9) and complement-dependent cytotoxicity (CDC). ADCC can be improved by complement receptor 3 (CR3) that bind to the iC3b. This enhances the high-affinity receptor for IgG (FcγR), leading to the induction of CR3-dependent cellular cytotoxicity (CR3-DCC) [11]. Previous studies have shown that β-glucan (βG) might act as an adjuvant for antitumor monoclonal antibodies by enhancing the leukocyte action against iC3b-coated tumor cells [14]. However, the success with βG therapy (previously suggested as an adjuvant to monoclonal antibodies) is limited due to natural antibodies and tumor escape (the ability of tumors to evade destruction by the immune system). It was thus hypothesized that the βG response could be improved by the administration of monoclonal antibodies together with βG [14]. Results concluded that the combined treatment with βG and monoclonal antibodies produced significantly higher tumor regression in models of mammary and hepatic tumors [15].

Application and clinical trials involving monoclonal antibodies in cancer immunotherapy

Monoclonal antibodies directed at surface proteins have revolutionized cancer treatment in the last decade. More than 100 monoclonal antibodies are currently being evaluated in clinical trials for a variety of cancers [15]. Cetuximab targets the epidermal growth factor family receptors and was approved by the Food and Drug Administration (FDA) for colorectal cancer. Bevacizumab (FDA approved for colorectal cancer immunotherapy) targets vascular endothelial growth factor (15). Rituximab (rituxan and mabthera) has received FDA approval [16] for non-Hodgkin's lymphoma treatment [17]. Rituximab recognises the transmembrane protein CD20 expressed on normal and malignant B lymphocytes [16]. Trastuzumab recognises and targets human epidermal growth receptor 2 (HER-2/neu) implicated in breast cancer treatment [15]. Gemtuzumab (attached to a cell toxin ozogamici) is directed against the CD33 surface antigen expressed [15] on approximately 90% of myeloblasts [18]. Once gemtuzumab attaches to the cell, ozogamicin enters the cell and destroys it by intercalation with deoxyribonucleic acid. These treatments have less frequent- and severe side effects (7) when compared to conventional chemotherapies namely decarbazine (DTIC) and tamoxifen [15].

In addition, bispecific monoclonal antibodies have been developed exerting dual specificities against TAAs and surface antigens on immune effector cells and direct cytotoxic cells to lyse neoplastic target cells [7]. The bispecific monoclonal antibody has been shown to promote target tumor cell [7] lysis by cytotoxic T lymphocytes (CTL) *in vitro* and *in vivo* in preclinical models at nanomolar concentrations [19].

Cytotoxic T-lymphocyte associated protein 4

Cytotoxic T lymphocyte associated protein 4 (CTLA4) is a major negative regulator of the immune system (20). CTLA4-blocking monoclonal antibodies activate antitumor T cells by obstructing the negative regulation of the T cell's function. Preclinical studies showed that anti-CTLA4 antibodies induced a regression in some murine tumors, with a lower threshold for inducing cytotoxic effects on cancer cells [20]. Blocking CTLA4 may result in a decrease of the activity of treg cells (dominant suppressor cells [21] with a crucial role in regulating autoimmune reactions in peripheral tissues and may allow for a lower threshold) [20].

Two CTLA4-blocking monoclonal antibodies are well-known and already in clinical trails [7]. The first report of CTLA4-blocking monoclonal antibodies used in humans detailed the infusion of ipilimumab [22] (previously known as MDX010) [7] to patients who were diagnosed with melanoma [23, 24]. Ipilimumab was combined with several other drugs including glycoprotein 100 (gp100), a monoclonal anti-Melan A antibody (MART-1), tyrosinase peptide vaccines and DTIC chemotherapy [25]. The combination of ipilimumab and DTIC chemotherapy revealed that clinical responses are higher in the combination group when compared to administrating ipilimumab alone.

Immunotherapeutic research has shown multiple benefits in the use of human monoclonal antibodies. Advantages include reduced or absence of cross-reactivity in normal human tissue, detection of polymorphic antigenic epitopes, enhanced interaction with immune effector cells of the host, reduced formation of immunocomplexes that may cause immunosuppression, as well as reduced-allergic and anaphylactic reactions in patients [7].

Cytokines and cancer immunotherapy

Certain cytokines induce innate or acquired immune responses directed against tumor cells [8]. Type I cytokines (tumor necrosis factor (TNF) and interferon-gamma (IFN-γ)) are part of the T helper 1 immune responses and mainly induce cell-mediated immunity, while type II cytokines (IL-4, IL-5, IL-6, IL-10 and IL-13) promote humoral immunity against tumors or immune changes to tolerance [25].

Various members of the TNF family induce apoptosis of cancer cells and contribute to tumor immunity [25,26]. The anti-metastatic property of NK cells [26] against TNF-related apoptosis-inducing ligand (TRAIL) is partly dependent on TRAIL [27]. This protective property of TRAIL is in turn dependent on IFN-γ [26]. TRAIL suppresses sarcoma formation similar to IFN-γ [27, 28].

Type 1 IFN includes 13 functional subtypes of IFN-α, IFN-β and IFN-ω. IFN-γ plays an important role in tumor recognition and elimination by the immune system. Type I INF is already in clinical use for cancer therapy, however, the mechanism is not fully understood; nor is it clear why some tumors are more responsive than others to IFN treatment. In some instances IFN works directly on the tumor cells and in other instances IFN exerts its function indirectly by inducing immunity to suppress the primary tumor and metastases [26].

IFN-α has antitumor activity in mouse- and human cancers. IFN-α promotes cytotoxic killing activity and proliferation of NK cells [26]. This is particular important in chronic meylogenous leukaemia treatment. In addition, IFN-α increases antigen presentation, immune surveillance and T cell-mediated killing of neoplastic cells.

Induction of NK cell activation is an alternative strategy using cytokines to eliminate cancer. This can be achieved by cytokines such as IL-21 and IL-12. IL-21 promotes maturation of human multipotent bone marrow progenitors and induces activation of NK cells. IL-21 might hold therapeutic potential in immunotherapy, since it stimulates NK cell- and T cell activity [26]. IL-21 leads to increased cytotoxicity and cytokine production when the NK cells are already activated. IL-21 treatment *in vivo* resulted in activation and maturation of NK cells. An increase in NK cell mediated immunity was also observed [26]. IL-12 plays a crucial role in the interaction between adaptive- and innate immunity. IL-12 is produced by phagocytic cells and dendritic cells. IL-12 acts on T cells, NK cells and causes Th1-cell differentiation leading to the production of IFN [26]. The effector cells required by IL-12 for the antitumor activity include NK cells, NKT cells, $CD4^+$ and $CD8^+$ cells. IFN-γ and TNF promote the production of chemokine (C-X-C motif) receptor 3 ligands (CXCR3) [26]. CXCR3 ligands and other chemokines, for example the IFN-inducible protein-10 [26], as well as monokine induced by gamma-interferon (Mig) chemokines affect the differentiation of newly formed vessels [29].

Application and clinical trails involving cytokines in cancer immunotherapy

IFN-α is used in over 40 countries for treatment of more than 14 different types of cancer. These cancers include haematological cancers (hairy cell leukaemia, chronic myeloid leukaemia and various B- and T cell lymphomas) and solid cancers (melanoma and various types of sarcoma) [26]. Reports suggest that IFN-α immunotherapy is more effective for haematological malignancies when compared to the outcome of solid tumor treatment [26].

TNF antagonist treatment on its own and in combination with chemotherapy commenced recently for solid tumors or for cancer cachexia [26]. Preliminary reports of breast cancer patients revealed no evidence of response and no

significant evidence [26] of toxic levels. Systemic TNF treatment for inoperable sarcoma lacked response levels and severe toxic levels were observed. However, adverse side effects of TNF-α including hypotension, vascular leakage, fever and neurotoxicity were observed [10].
Administration of IL-12 resulted in tumor growth inhibition and suppressed metastasis [3] in animals. However, clinical trails involving IL-12 administration had limited success due to the systemic cytotoxicity caused by IL-12 to organs including the lymphohematopoietic system [8]. Subcutaneous administration of IL-12 for renal cell cancer patients resulted in less toxicity [26]. Systemically administered IL-2 resulted in an impact on already established lung metastases in mice. When IL-2 was used in conjunction with lymphocytes it inhibited growth of lung- and liver cancer. Combination therapy with cytokines namely IL-2 and IL-8 proved to have potential in preclinical trails in mice tumor models [26].

Since IL-2 may lead to the stimulation of T cell activity, it has paved the way for new possibilities of stimulating the immune response against cancer cells [30]. However, adverse side effects of IL-2 namely hypotension, vascular leakage, respiratory difficulty, nausea, emesis, diarrhoea, myalgias, arthralgias, myocardial infarction, myocarditis, infection, renal failure and bowel infarction were noted [10].

Vaccines and cancer immunotherapy

Vaccines involve an active immunotherapeutic approach [5,31] in which an immune response is elicited rather than passively supplied to the body [5,31,32].

The optimal tumor antigen should have homogenous expression throughout the tumor with little or no expression in non-cancerous tissue and should be expressed on the surface of several types of tumors [33]. Several characteristics of tumor antigens make them appealing targets for cancer vaccines namely, lack of pre-existing tolerance, differential expression on tumor cells, absent expression on normal cells and its role in tumorgenesis [34].

Different types of antigens or targets [34] are used in cancer vaccines depending on the type of cancer [35]. The approach of patient-specific vaccines is currently under investigation in clinical trails [31, 34]. In addition to antigens, heat shock proteins (HSP) can also be used to produce effective antitumor responses. HSP are produced as a result of heat, low sugar levels or in cellular stress situations. These proteins are involved in the correct assembly and folding of proteins [36]. Studies are still in progress involving HSP vaccines for liver, skin, colon, lymphoma, lung and prostate cancers. Research revealed that HSP-peptide complexes derived from a patient's tumor can be generated *in vitro* and elicit specific CTL responses without adjuvant usage. In addition, a study has indicated that mild, fever-like hypothermic conditions combined with tumor-derived HSP immunization significantly reduced tumor sizes [36].

The advantage of cancer vaccines is thus the ability to target the surface and intracellular tumor antigens by stimulating immune responses with the potential of long-term longevity [34].

Discussion

Tumor development is influenced by its microenvironment. There is accumulated evidence that the tumor environment is a hypoxic environment under which the normal immune cells do not function properly when compared to normal oxygen tension [37]. Recently, results were obtained that anaerobic microbes preferentially colonize hypoxic regions and cause cell lysis. This may lead to new possibilities in immunotherapy, especially for breast cancer where there is renewed interest of microbes destroying breast tumors [38].

Future studies to optimize dendritic vaccination include determining the preferred dendritic cell type and the optimal antigen loading technique. This will confidently result in a standardized dendritic cell vaccination [39]. It is; however, clear that at this time vaccinations and immunotherapy in general should be combined with other known treatments to exert an optimal effect in disease.

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