ISSN 1466-2523

Animal Health Research Reviews **5**(2); 227–234
DOI: 10.1079/AHR200473

Cell-based cancer gene therapy: breaking tolerance or inducing autoimmunity?

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Abstract

This review examines the mechanisms involved in anti-tumor immunity and how peptides present in many tumor types (tumor-associated antigens) are recognized by T cells from tumor-bearing cancer patients. Tumor-associated antigens are derived from proteins that are also expressed in normal cells. It is predicted that immune responses to such peptides will be compromised by self-tolerance or that stimulation of effective immune responses will be accompanied by autoimmunity. We also consider that the immunity induced against two autoantigens, which are highly conserved in vertebrates, involve qualitatively different mechanisms, such as the production of antibodies and cell-mediated immune responses. However, both pathways lead to tumor immunity and identical phenotypic manifestations of autoimmunity. Appropriate selection of the optimal tumor antigen is critical for the induction of an anti-tumor immune response. Thus, we stress that the methods for antigen presentation using dendritic cells play a critical role in the development of tumor vaccines, to break immune tolerance and induce a strong immune response against them. The viability and feasibility of expansion of canine dendritic cells from bone marrow and peripheral blood *ex vivo* for the treatment of spontaneous cancers in dogs is also discussed.

Keywords: dendritic cell; gene therapy; melanoma; autoimmunity; immunotherapy; tolerance; canine; peripheral blood

Anti-tumor immunity

The observation, in 1893, that spontaneous regression of sarcomas could occur in patients with acute bacterial infections led to the hypothesis that the bacterial infection stimulated the immune system, which was then able to mount a response to destroy the tumor (Chamberlain and Kaufman, 2000). Therefore, the initial question was whether the immune system is capable of discriminating between transformed cells of the tumor (non-self) and their normal cell counterparts (self). It is now well established that a physiological function of the immune system is to prevent the growth of tumors and to eliminate them. Regardless of the source of the tumor antigen, tumor rejection is mediated by cytotoxic T lymphocytes (CTL), which recognize peptides derived from these antigens. CTL responses are dependent on the ingestion of tumor cells, or their antigens, by professional antigen-presenting cells, which are best represented by dendritic cells (DCs), and the subsequent presentation of processed peptide epitope to T cells (Wang, 2002) (Fig. 1). However, tumor cells use mechanisms to avoid detection and destruction by the immune system, such as the down-regulation of cell-surface HLA class I expression, the down-regulation of tumor antigen expression or the selection of tumor variants that are negative for the antigen, lack of co-stimulatory molecules on tumor cells, and the production of

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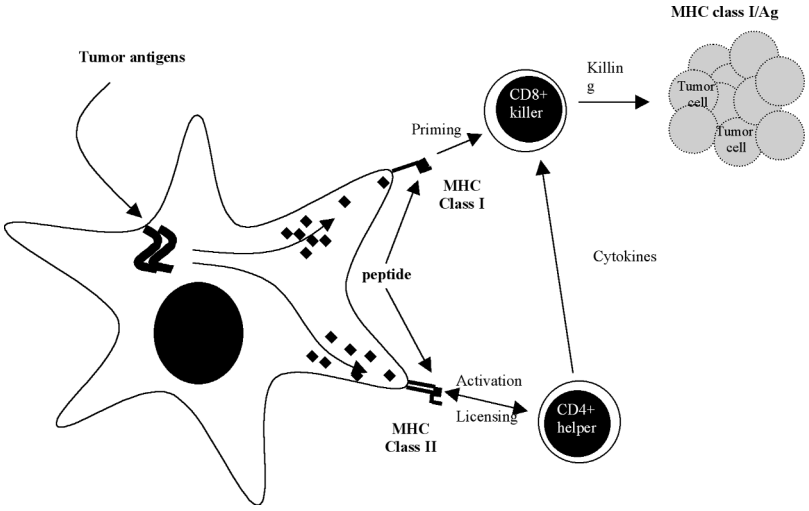

Fig. 1. Effect of CD4+ and CD8+ T cells against tumor cells. CD8+ and CD4+ T cells express clonally distributed receptors that recognize fragments of antigens (peptides) associated with MHC class I and II molecules respectively. Soon after encountering a danger signal, the efficiency of antigen uptake, intracellular transport and degradation and the intracellular traffic of MHC molecules are modified. Antigen degradation and peptide loading onto MHC molecules occurs intracellularly in antigen-presenting cells, including dendritic cells (DC). Strict compartmentalization of MHC class I and II biogenesis results in the loading of exogenous and endogenous antigens on MHC class II molecules in the endocytic pathway and the selective loading of endogenous, but not exogenous, antigens on MHC class I molecules in the endoplasmic reticulum. This model accounts, at the effector level, for the selective killing of tumor cells by MHC class I-restricted CD8+ CTLs. The *in vivo* relevance of DC maturation in T-cell priming is particularly clear in the case of CD8+ T cell priming. Despite several examples of direct priming of CTL by DCs in the absence of CD4+ T cells, several *in vivo* CD8+ T cell responses are dependent on CD4+ T-cell help. In addition, CD4+ T cells may also convert induction of CD8+ T-cell tolerance into priming in cases of constitutive cross-presentation of peripheral self-antigens.

immunosuppressive cytokines [including transforming growth factor β and interleukin (IL) 10] (Platsoucas *et al.*, 2003). The identification of tumor-associated antigens (TAA) has enabled the expansion of efforts to develop specific anti-tumor immunotherapies that are capable of overcoming peripheral tolerance against poorly immunogenic, non-mutated tumor antigens (Overwijk *et al.*, 1999). Thus far, significant advances have been made in the development of tumor vaccines and adoptive immunotherapy approaches, which suggest that further effort may lead to effective therapeutic interventions in a number of cancers.

T cells (central and peripheral tolerance, and ignorance)

The importance of T-cell-mediated anti-tumor immunity has been demonstrated in both animal models and human cancer therapy. However, the context in which an antigen is presented shapes the nature of the immune response, and can result in B-cell activation, T-cell activation or immune tolerance. The normal immune system is capable of reacting to a vast variety of microorganisms, but it reacts poorly to an individual's own (self) antigens (Zeuthen *et al.*, 1998).

Under normal circumstances, tolerance to self-antigens is generated by exposure of lymphocytes to these antigens in lymphoid tissues. Lymphocytes with T-cell receptors specific for an individual antigen are activated to generate an immune response by exposure to this antigen. However, these lymphocytes may be functionally inactivated or killed (tolerance), or may not react in any way (ignorance or anergy). Self-antigens are encountered constantly, but if immature T cells in the thymus recognize a self-peptide bound to a major histocompatibility complex (MHC) molecule, the lymphocytes die by apoptosis (negative selection – central tolerance). In the peripheral organs, if a mature T cell, which is not specific for self-antigens, recognizes self-antigens without co-stimulators, it leads to the functional inactivation of the T cells. Alternatively, the T cells may use the inhibitory receptor (CTLA-4) to recognize co-stimulators, leading to T-cell anergy and death as a result of continual active suppression of the self-reactive lymphocytes (Abbas, 2003). T lymphocytes with self-destructive capacity are often found in healthy individuals, suggesting the existence of efficient control mechanisms that prevent autoimmune diseases. This beneficial effect, however, may in turn be responsible for tumor immune evasion. Indeed, tumors often continue to grow in patients despite the presence of T cells specific for the respective tumor antigens (Arnold, 2002).

Central tolerance accounts for T-cell tolerance against common surface markers, but tissue-specific or developmentally regulated antigens are not presented to T cells

https://doi.org/10.1079/AHR200473 Published online by Cambridge University Press

within the thymus, so that potentially self-reactive T cells specific for these antigens could migrate to the periphery, making the development of central tolerance incomplete. Even though the immune system has a variety of mechanisms to induce peripheral tolerance, this process does not completely exonerate the peripheral, autoreactive T cells. It has been proposed that these autoreactive T cells could be responsible for generating immune responses against tumors. Overexpression or three-dimensional irregular expression of self-proteins on tumors may permit the immune system to mount an effective response against otherwise normal self-protein (Wan *et al.*, 2001).

Tumor-associated antigens (TAA)

While whole tumor cells remain a potent vehicle for generating anti-tumor immunity, the cloning and characterization of TAA has resulted in increased efforts to develop specific anti-tumor immunotherapeutics. Lineage-specific or differentiation antigens and their highly antigenic subunits have emerged as better candidates for the development of effective tumor vaccines (Ward *et al.*, 2002). Among the TAA are the differentiation antigens, which are non-mutated self-antigens expressed specifically by both normal and transformed cells of a particular cell type. There are four groups of TAAs, classified according to where the antigens are derived. Group 1 consists of tumor-specific transplant antigens, genes with mutations not related to the process of transformation and, as such, specific to each patient (specificity of expression and lack of central tolerance to the antigen); group 2 consists of antigens that have mutated as part of the transformation process, as mutated oncogenes (minimal tolerance and cross-reactivity to normal proteins, thereby undetected autoimmunity); group 3 consists of antigens that are shared with normal cells with extremely limited tissue expression (they are expressed in immunologically privileged sites, there is no tolerance, and they elicit strong responses); and group 4 consists of antigens which are shared with normal cells and have widespread, but nevertheless tissue-specific, expression (there is some level of tolerance and a poor immune response) (Gilboa *et al.*, 1998). Recent years have seen the identification of melanoma-associated antigens, which are recognized by CTL and can attack melanoma cells in an HLA-restricted and tumor antigen-specific manner. These antigens belong to three main groups: tumor-associated testis-specific antigens (MAGE, BAGE, GAGE and PRAME), melanocyte differentiation antigens (tyrosinase, melan-A/MART-1, gp100, TRP-1 and TRP-2) and mutated or aberrantly expressed antigens (MUM-1, CDK4, β-catenin, gp100-ina, p15 and N-acetyl glucosaminyl transferase V) (Zeuthen *et al.*, 1998). Tumor cells express a number of potentially immunogenic TAA. However, while melanocyte differentiation antigens expressed by normal melanocytes and melanoma cells can be recognized by the immune system, the overall immunogenicity of tumor cells remains, in general, low.

Autoimmunity and cancer immunotherapy

Self-antigens, in the form of differentiation antigens, are commonly recognized by the immune system on melanomas and other cancers. The immune repertoire includes T cells and B cells that can recognize autologous cancer cells. While this repertoire can be directed against self, and in some cases altered-self (mutations), priming immune responses against self-antigens is quite difficult. Malignant tumors may escape rejection by the immune system because they can induce a state of immunological tolerance, mediated by tumor antigen-specific suppressor T cells (Eck and Turka, 2001). However, a large set of peptide antigens presented by class I MHC molecules on human and murine melanomas and recognized by $CD8^+$ T cells have been defined, which is evidence that peripheral tolerance is incomplete (Fig. 1). These peptides represent attractive candidates for the development of therapeutic and/or prophylactic approaches to treating this type of cancer. The majority of the peptides presented by this type of tumor, and recognized by T cells from multiple patients, arise from proteins that are also expressed in normal melanocytes. It is, therefore, expected that immune responses to such peptides will be compromised by self-tolerance or that the stimulation of effective immune responses will be accompanied by autoimmune responses, such as vitiligo (Engelhard *et al.*, 2002).

Biological response modifiers (BRM) improve the body's ability to fight cancer by immunostimulation. Although a century has passed since the first attempt was made to stimulate the host immune system against cancer, only the past decade has witnessed the scientific use of BRMs (Gupta and Kanodia, 2002). Recent advances in tumor immunology have enabled the development of specific agents targeted against cancer cells. BRMs include monoclonal antibodies, interferons, interleukins, tumor necrosis factor (TNF), colony stimulating factors and anticancer vaccines. Monoclonal antibodies directed against tumor-specific agents have been approved for the treatment of breast cancer (trastuzumab) and non-Hodgkin's lymphoma (rituximab), and for the diagnosis of certain cancers (oncoscint). Interferons are indicated for the treatment of certain leukemias and Kaposi's sarcoma, to inhibit tumor proliferation and angiogenesis. Interleukin 2 is the most widely studied interleukin, and is used for immunostimulation in metastatic renal cell carcinoma and malignant melanoma. Hematopoietic growth factors are often combined with chemotherapy and radiotherapy, to restore bone marrow function and treat

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complications such as infection and bleeding. Thalidomide, which suppresses TNF-α production and has anti-angiogenic properties, is currently under evaluation in several cancer types. Various anticancer vaccines are in development, using tumor cells, carbohydrates, peptides and heat-shock proteins as antigens. DNA-based vaccines and the use of recombinant bacteria and viruses to deliver antigens, or the DNA coding for them, are also being investigated. However, the optimum choices of antigen, delivery vector, adjuvant and administration regimen for some of these BRMs are still under investigation (Gupta and Kanodia, 2002). To elicit anti-tumor immune responses, various cell types have been employed as cellular adjuvants with tumor antigens, and recently several groups have shown that DCs, cultured with tumor lysates, tumor antigens or peptides eluted from tumor cells, induced significant anti-tumor immunity *in vivo* (McArthur and Mulligan, 1998). DC-based vaccines are more effective than naked DNA-based vaccines at eliciting anti-tumor immunity, in both prophylactic and therapeutic models. This suggests that using DCs transfected with DNA containing a TAA gene may be superior to the use of peptide-pulsed DCs and naked DNA-based vaccines for immunotherapy and may provide an alternative strategy for tumor vaccine design (Yang *et al.*, 1999).

Dendritic cells

DC are bone-marrow-derived leukocytes that are the most potent antigen-processing cells, capable of sensitizing T cells to new and recall-specific antigens. DCs have a branched tree-like morphology. They are responsible for the transport of antigens to lymph organs and the presentation of antigen to T cells. They engulf external antigens by macropinocytosis and endocytosis. DCs process antigens proteolytically, by hydrolysis of the proteins within their lysosomes. DCs then present the processed antigen, attached to an MHC I and/or MHC II complex on the surface of the cell, to T cells in the lymph node (Clark *et al.*, 2000). T cells are activated by several molecules present on the surface of DCs, such as co-stimulatory molecules B7.1 and B7.2, which activate specific T-cell subsets (Fong and Engleman, 2000). As DCs migrate from lymph pathways to the lymph node they mature and lose their ability to take up antigen and change shape. DCs are thought to arise from myeloid and lymphoid lineages, thus conferring functional and spatial differences on mature DCs (Banchereau *et al.*, 2000). In the presence of defined cytokines, mature DCs of myeloid origin can be cultured *ex vivo*, from isolated $CD14^+$ blood monocytes or from bone marrow-derived $CD34^+$ cells, for clinical applications (Fig. 2).

Dendritic cell lineage

Lymphoid and myeloid DCs exist in various stages of differentiation. DCs are continuously produced from hematopoietic stem cells within the bone marrow and can be divided into subsets according to surface marker expression, specialized functions and tissue distribution

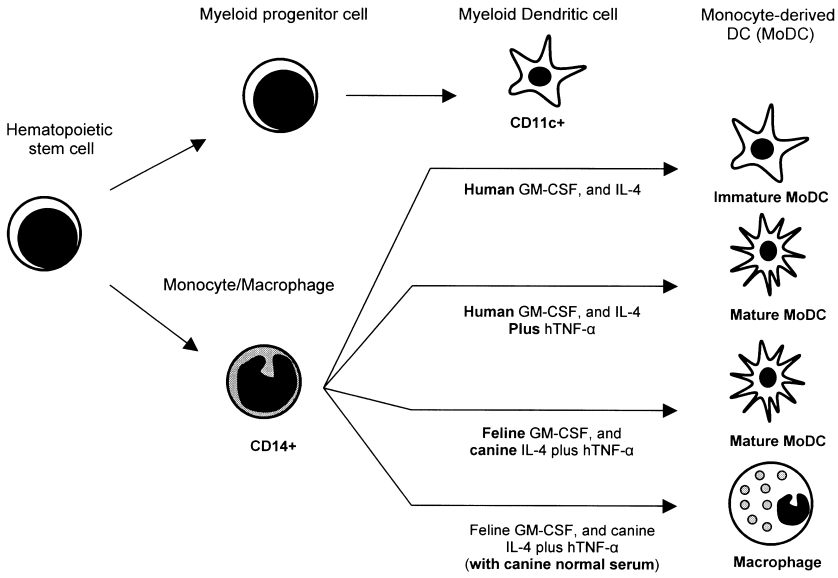

Fig. 2. Generation and expansion of canine dendritic cells (DCs) from peripheral mononuclear cells using different cytokine cocktails. Peripheral blood mononuclear cells were isolated from 20, 30 and 40 ml of heparimized blood by density-gradient centrifugation with Ficoll 1077 g/ml, and cultured at 2×10^6 /ml in a six-well tissue culture plate with recombinant human, feline or canine granulocyte-macrophage colony stimulating factor (GM-CSF), human or canine IL-4 and human TNF-α (hTNF-α) for 7 days (J. C. Rodriguez-Lecompte, K. Linher, J. Bramson, S. Kruth, J. P. Woods and J. Gauldie, unpublished).

https://doi.org/10.1079/AHR200473 Published online by Cambridge University Press

(Rescigno, 2002). Immature DCs are found in blood, non-lymphoid and lymphoid tissues, whereas mature DCs are primarily found in lymphoid tissues (Banchereau *et al.*, 2001). In humans, two types of DCs have been described: myeloid, which include epidermal DCs (Langerhans cells) and dermal or interstitial DCs, which are also found in other peripheral tissues. Immature myeloid DCs can be found in circulating blood or can be generated from blood precursors (monocytes) during transit from peripheral tissues (Sozzani *et al.*, 2000). The key feature in the development of myeloid DCs is the functional switch from the immature to the mature stage. The special functional properties of myeloid DCs facilitate antigen transport from the periphery to lymphoid organs and, therefore, efficient monitoring of the antigen environment by the immune system (Ludewig, 2003). Peripheral DCs are surveillance cells that capture antigen, which is then processed and, following migration of the DC to the T-lymphocyte areas of draining lymph nodes, the antigenic epitope is presented to lymphocytes within the context of the MHC molecules (Clark *et al.*, 2000).

Dendritic cells as activators of T-cell immunity

DCs have several functions in both innate and adaptive immunity. In addition, there is increasing evidence that DCs *in situ* induce antigen-specific unresponsiveness or tolerance in central lymphoid organs and in the periphery. In the thymus, DCs generate tolerance by deleting self-reactive T cells. DCs have receptors for the efficient uptake of proteins and fragments of dying cells, which they then process and present, to induce tolerance to self antigens in peripheral lymphoid organs. The incorporated antigen is presented on MHC class I and II molecules. In a mouse model, exposure of DCs to low doses of antigen can result in deletion of the corresponding antigen-specific T cells. When challenged subsequently, even in the presence of strong adjuvants, the T-cell population will remain unresponsive. In contrast, if a DC maturation stimulus is co-administered with the antigen, immunity develops, including interferon γ-secreting effector T cells and memory T cells. There is also new evidence that DCs can contribute to the expansion and differentiation of T cells that regulate or suppress other immune T cells. It is possible that distinct developmental stages and subsets of DCs and T cells account for the different pathways to peripheral tolerance, which include deletion or suppression of self-reactive T cells (Fazekas de St Groth, 2001). The interaction between DCs and T lymphocytes involves several ligand–receptor pairs, including MHC, adhesion, and co-stimulatory molecules. DCs are more than a simple activator or repressor switch of the immune response; they also contribute significant polarizing influences on T-helper cell differentiation (Moser, 2003). Monocyte-derived DCs (DC1) secrete large amounts of IL-12, and tend to favor the development of Th1 responses, which stimulate cell-mediated immunity designed to protect against intracellular organisms. Lymphoid DCs (DC2) produce not IL-12 but other cytokines, such as IL-4, which favors the development of Th2 cells, and this stimulates antibody-mediated immune responses against extracellular pathogens. However, the mechanisms by which DCs control the Th1/Th2 balance *in vivo* appear to be more complex than previously supposed. It has been accepted that influences such as the localization of DC subsets, the duration of DC activation and environmental, antigen and tissue factors may contribute to the commitment of T helper cells *in vivo* (Maldonado-Lopez and Moser, 2001).

Dendritic cells and anti-tumor immunity

In recent years there has been much debate regarding whether tumor cells prime naive T cells directly, or whether tumor-derived antigens must first be taken up by antigen-presenting cells, processed and then presented to T cells. Tumors adapt to and alter the immune system by exhaustion, ignorance, tolerization, inhibition of antigen-specific cells, or antigen loss. In addition, tumor antigens are, in general, weak antigens. They are often masked or sequestered inside the tumor cell, or are expressed at barely detectable levels (Gunzer *et al.*, 2001). However, strong evidence is accumulating to indicate that tumor cells in humans and animals are recognized, in general, as non-self by the immune system and that they can induce an immune response, leading to their elimination (Platsoucas *et al.*, 2003). Direct priming of T cells by tumor cells transfected to express co-stimulatory B7 molecules, growth factors (IL-2) and even MHC class II is promising. However, the characteristics of lymphocyte recirculation probably do not allow naive T cells to encounter tumor cells outside of lymphoid tissue (Schweighoffer, 1996). The initiation of every cellular immune reaction against tumor antigens must involve presentation of the antigen to T cells, in order to activate them and drive them into clonal expansion. Therefore, DCs, the most powerful antigen-presenting cells, are an ideal candidate to generate effector cells specific for the neoplastic disease in cancer patients. Additional requirements for T-cell activation include the engagement of co-stimulatory receptors on T cells, adequate types and concentrations of T-cell-activating cytokines and T-cell-attracting chemokines, and maintenance of the activation signal for a sufficient period of time (Gunzer and Grabbe, 2001). Lineage-specific or differentiation antigens appear to be the optimal candidates for the development of anti-tumor immune responses because they are expressed on all tumor cells. The mode by which the

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DC presents the processed antigen to the immune system is critical to the success of a candidate tumor vaccine. DCs can be modified to express genes of specific tumor antigens, in order to activate both helper and cytotoxic T cells. Novel vaccines have been engineered to generate specific immune responses and objective clinical responses with minimal toxicity in phase I/II trials (Schreurs *et al.*, 2000). Advances in gene transfer technology, tumor immunology and improved methods of monitoring specific anti-tumor immune responses permit the hope that tumor vaccines will reach the clinic, at least in malignancies which prove resistant to traditional therapy, such as melanoma and renal cell carcinoma. Previously, the isolation of DCs was problematic, but it is now possible to isolate and expand DC populations *in vitro* as well as to manipulate them before returning them to a patient in order to induce tumor immunity (Fig. 2).

Dendritic cell vaccines for cancer

The objective of active immunotherapy using DC-based cancer immunotherapy is to reverse immune tolerance to the tumor. Introducing DCs cells loaded with tumor antigens to patients allows the immune system to respond appropriately to self-antigens, and primes specific anti-tumor immunity through the generation of effector cells that attack and lyse tumors (Engleman and Fong, 2003). DC-based vaccines represent an attractive approach to cancer immunotherapy for three main reasons: (i) exploiting the adjuvant nature of DCs makes good biological sense, as class I-restricted antigens must first be processed by DCs to activate the CTL arm of the immune system; (ii) DCs are capable of activating naive T cells, $CD4^+$ T-helper cells as well as $CD8^+$ CTL, and (iii) preparation of a DC vaccine format is a relatively simple and clinically manageable process (Gilboa *et al.*, 1998). The identification of MHC class I-restricted tumor antigens has generated a resurgence of interest in immunotherapy for cancer. However, recent studies suggest that therapeutic strategies that have focused on the use of $CD8^+$ T cells and MHC class I-restricted tumor antigens may not be effective in eliminating cancer cells in patients. Novel strategies have been developed to enhance T-cell responses against cancer by prolonging antigen presentation by DCs to T cells, and by the inclusion of MHC class II-restricted tumor antigens. Identification of MHC class II-restricted tumor antigens, which are capable of stimulating $CD4^+$ T cells, provides increased opportunity for developing effective cancer vaccines and aids our understanding of the host immune responses against cancer (Wang, 2002).

DCs are currently used in clinical studies to induce immunity against infectious disease and malignant cells. The existence of multiple DC subsets suggests that the type of DC may affect the immune response induced. The vast majority of DCs used in experimental mouse tumor models are derived from bone marrow progenitors. In contrast, most *in vitro* studies, as well as *in vivo* human studies, involve the use of DCs generated from adherent peripheral blood-derived monocytes in the presence of cytokines, such as granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-4. These cytokines have been found to induce the maturation and enhance the viability of DCs isolated from peripheral blood (Engleman, 2003) (Fig. 2). Isolated DCs loaded with tumor antigen *ex vivo* and administered as a cellular vaccine have been found to induce protective and therapeutic anti-tumor immunity in experimental animals (Timmerman and Levy, 1999). Considering that most therapeutic cancer vaccine trials are performed in patients at the end stages of their clinical disease, it is likely that at earlier stages of cancer these vaccines will prove more effective (Berinstein, 2003).

Use of recombinant adenovirus for dendritic cell transduction

Immunotherapeutics using DCs as antigen-presenting delivery vehicles for cell-based vaccines have already improved patient outcome against a wide range of tumor types. Several tumor antigen loading strategies for the induction of anti-tumor immunity via DCs exist, such as peptides, protein, receptor-mediated, liposome-mediated, peptide-mediated, recombinant bacterial toxin-mediated, whole tumor cell antigens, tumor-derived exosomes, tumor-derived RNA and tumor-derived DNA (Zhou *et al.*, 2002). The choice of vector will depend on the application required and on the susceptibility of the target cells to transduction (Bramson and Wan, 2002). E1-deleted adenoviral (Ad) vectors are replication-incompetent and are used in recombinant viral vaccines. They can produce transient, high-level expression of the transgene product and there is no risk of community exposure. They are particularly useful for studies in which the tumor is genetically modified to act as a vaccine, permitting both humoral and cellular immune responses to be generated within a few days (Brenner, 2001). Following DC transfection with Ad vector, increased expression of co-stimulatory and maturation molecules occurs (Foley *et al.*, 2001) and high antigen expression and processing results in presentation of antigen epitopes through both class I and II

We have developed a recombinant Ad vector to deliver antigen genes as an anti-tumor vaccine, either directly or in combination with DCs. We have shown that both direct injection with Ad vector expressing a defined TAA, such as the self melanoma-associated antigen gp100 (Adgp100), and the inoculation of DCs transduced *ex vivo* with this same vector can induce a T-cell-directed anti-tumor response. This represents an attractive and practical approach for the development of anti-tumor immunotherapeutics. Our results showed, in several murine tumor

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models, that vaccination using either strategy could induce antigen-specific, T-cell-mediated anti-tumor responses (Fig. 3). Foreign antigens and self-antigens have different requirements for effective induction of $CD4^+$ and $CD8^+$ cells to yield protective immunity. An Ad vaccine expressing the TAA human gp100 fails to directly induce significant immune responses, while DCs transduced *ex vivo* with Adhgp100 as a vaccine (DCAdhgp100) result in complete protection against murine melanoma challenge (Fig. 4).We have extended these preclinical studies to two clinical trials in canine and human melanoma. Human DCs derived from $CD34^+$ stem cells are grown under stimulation with GM-CSF, Flt3 ligand and TNF-α. Either bone marrow (BM)- or peripheral blood (PB)-derived canine $CD14^+$ DCs are grown under stimulation with stem cell factor, GM-CSF, IL-4 and TNF-α. We have demonstrated methods for the *ex vivo* expansion and characterization of canine DCs derived from bone marrow (S. Gyorffy, J. C. Rodriguez-Lecompte, J. P. Woods, R. Foley, S. Kruth, P. C. Liaw and J. Gauldie, unpublished) and PBMC (Rodriguez-Lecompte *et al.*, 2003, unpublished). The purified and enriched preparations of DCs are transduced for 24 hours with Adhgp100 and/or AdhTRP-2 and the tumor-bearing host is inoculated with three doses of cell-based vaccine over a 3-month period. Clinical monitoring shows this to be a safe method for inducing tumor antigen-specific T-cell immunity and shows promise for development as a potent means of inducing therapeutic immune responses against some cancers.

Conclusions

Since tumor cells are considered poorly immunogenic, mainly because they express self-antigens in a non-stimulatory context (immunotolerance), the environment of the tumor's cells may have to be modified to become stimulatory by using immunological adjuvant. Human and animal studies have demonstrated that DCs transduced with replication-incompetent adenovirus vectors expressing tumor antigen can efficiently mediate the induction of antitumor immunity. DC-based vaccine induces immunity against autologous tumor-associated antigen and also stimulates CTL-mediated antitumor responses, which can proceed without apparent development of adverse autoimmunity against normal tissue. Further optimization of cancer vaccines and large clinical studies are required to pursue this approach for realist therapeutic intervention.

Acknowledgments

We thank Dr Gail Martin for careful and critical reviews of the manuscript and Jane Ann Smith for technical assistance. This work was supported by the Canadian Institutes of Health Research (CIHR), NCIC and the Canadian Network for Vaccines and Immunotherapeutics (CANVAC).

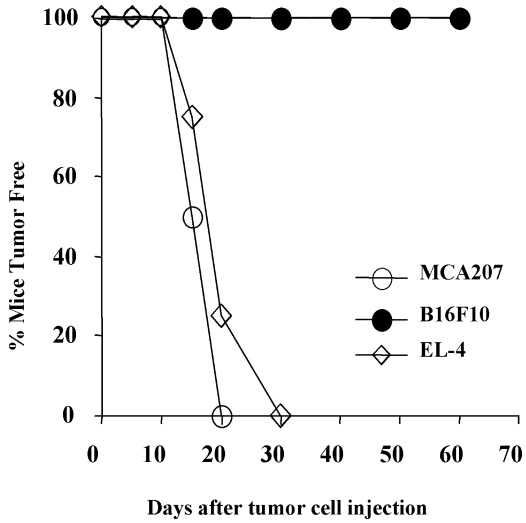

Fig. 3. Dendritic cells transduced with adenovirus encoding human gp100 (DCAdhgp100)-induced anti-tumor immunity is tumor-specific. $CD8^{-/-}$ mice were immunized subcutaneously with 1×10^6 DCAdhgcp100 and challenged with B16F10, EL4 or MCA207 melanoma cells. Fourteen days after immunization, tumor formation was monitored twice a week (Wan *et al.*, 2000).

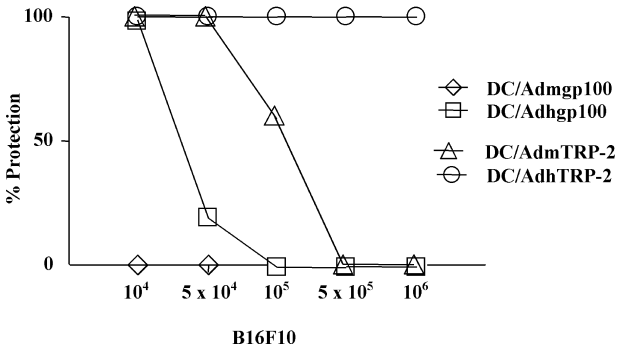

Fig. 4. DC/Ad vaccines encoding xeno-antigens enhance protective immunity against self tumor antigens. C57BL/6 mice were immunized with DC/Ad vaccines encoding tumoral, autologous (DC/Admgp100, DC/AdmTRP-2) and xeno-antigens (DC/Adhgp100, DC/AdhTRP-2) subcutaneously on either hind flank. Fourteen days later the mice were challenged subcutaneously with 10^4, 5×10^4, 10^5, 5×10^5 or 1×10^6 B16F10 murine melanoma cells. Tumor growth was then monitored several times a week for 60 days. Each group represents pooled data from at least three experiments (L. Patton, J. Wan and J. Gauldie, 2004, unpublished).

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