

Dendritic cell based vaccines for immunotherapy of cancer

Review Article

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Summary

Researchers and clinicians have tried for decades to use specific and non-specific immunotherapy for the fight against cancer. Initial attempts were based on soluble immune mediators such as antibodies or cytotoxic proteins for the therapy of malignancies. Major improvements in our understanding of the induction and regulation of cellular immunity have now made it possible to generate effector cells in cancer patients that can specifically recognize and destroy malignant cells. Human tumors express a number of protein antigens that can be recognized by T cells, thus providing potential targets for cancer immunotherapy. Tumor antigens have to be presented to T cells in order to activate them and drive them into clonal expansion. This is done by antigen presenting cells (APCs). Dendritic cells (DCs) are professional APCs, which have an extraordinary capacity to stimulate naïve T cells and initiate primary immune responses. This established function of DCs has now offered the hope to apply DC-based immunotherapy for cancers. Pilot clinical trials of DC vaccination have established the safety and feasibility of this approach and have produced encouraging evidence of therapeutic efficacy. Importantly, significant advances in our understanding of the DC biology can be used to support the design of new vaccines in order to elicit effective cellular immune responses for the treatment of cancer. In this review, recent findings of DC immunotherapy for a variety of tumors including colon cancer have been discussed. Also, the development of DC-based vaccines in preclinical models of colorectal cancer as a prelude to clinical trials has been summarized.

I. Introduction

Colorectal cancer is one of the most common malignancies in men and women representing 10% of all cancer deaths in the United States with ~ 130,000 new cases diagnosed annually (Landis et al, 1998). Progression of the disease occurs through invasion of the colonic wall, involvement of regional lymph nodes, and distant metastasis. At the time of diagnosis, almost 50% of the patients have tumors invading through the bowel wall with spread to regional lymph nodes; in addition, 10% of the patients present with synchronous metastatic cancer to the liver. There have been important advances in our understanding of the biology and genetics of this disease, and if diagnosed early, colorectal cancer is curable. Surgery is associated with a 90% five-year survival rate in patients with tumors involving only the mucosa or submucosa. However, for majority of the patients having

metastasis of the disease in local lymph nodes and adjacent organs, survival is substantially worse (Greenlee et al, 2000). Systemic chemotherapy with 5-fluorouracil-based regimens or newer agents such as irinotecan has improved survival of these patients with high-risk disease (Moore and Haller, 1999). Despite this, only 60% of all patients diagnosed with colorectal cancer survive more than ten years. At present there is no therapeutic regime capable of curing unresectable metastatic disease. Clearly, more effective treatments are necessary for this disease.

Vaccines against infectious diseases are the success story of immunology. Smallpox has been eradicated and vaccination strategies have saved countless people from tetanus, polio, measles and hepatitis. Consequently, there is a hope for the generation of effective cancer vaccine(s). However, to date, human anti-tumor vaccination has not delivered on its promises. Reasons for failure include tumor immune escape

mechanisms, limited availability of tumor specific antigens, as well as failure to deliver tumor antigens in the right immunological context. Progress in immunology and molecular biology has provided technologies to detect an ever increasing choice of new tumor specific antigens. One of the most important issues is to deliver these tumor antigens in an effective way to induce immune responses in cancer patients. However, recent insights into the role of DCs as the pivotal APCs that initiate immune response may provide the basis for generating more effective antitumor immune responses in patients. Our understanding of the DC biology has opened new ways for the application of these cells for immunotherapy of cancer.

Immunotherapy is an attractive approach to cancer therapy. The aim of immunotherapy is to induce or increase the ability of the host to mount antitumor immune responses *in vivo*. Convincing evidence now exists that the effector cells of the immune system (T, B, and NK cells), when appropriately activated, are able to lyse tumor cells through specific recognition of tumor associated antigens (TAAs) (Rosenberg, 1996; Finn and Lotze, 1998). Although a variety of both humoral and cellular antitumor immune responses have been documented, T cells, and in particular CD8⁺ cytotoxic T lymphocytes, are likely to play an important role in antitumor immunity (Maeurer and Lotze, 1997). Identification of TAAs together with a better understanding of the mechanisms involved in the immune response against cancer, have given investigators tools to manipulate the immune system to induce an efficient immune response in the tumor bearing host (Pardoll, 2000; Van Gool et al, 2000; Borrello and Sotomayor, 2002; Drake and Pardoll, 2002).

II. What is a dendritic cell?

DCs were originally discovered as antigen presenting cells critical for the induction of primary T cell dependent immune responses (Steinman, 1991). DCs represent only 0.5% of blood leukocytes. Like other cell types within the immune system, they arise from a common CD34⁺ progenitor in the bone marrow whose expansion and differentiation is influenced by a variety of cytokine growth factors including stem cell factor, fetal liver tyrosine kinase-3 (Flt-3) ligand, IL-3, granulocyte/macrophage colony stimulating factor (GM-CSF), TNF- and TGF- (Shortman and Caux, 1997; Pulendran et al, 2001). Mature DCs have a distinct morphology characterized by the presence of numerous membrane processes that can take the form of dendrites, pseudopods, or veils. Morphologic features of DCs include high concentrations of intracellular structures related to antigen processing such as endosomes, lysosomes, and the Birbeck granules of Langerhans cells of the epidermis.

DCs are also characterized by abundant expression of molecules used for their specialized interactions with T cells. These include the antigen-presentation molecules CD1 and the class I and class II MHC proteins, the adhesion molecules CD11a (LFA-1), CD11b, CD11c, CD50 (ICAM-2), CD54 (ICAM-1), CD58 (LFA-3), and CD102 (ICAM-3), although all of these markers can also be found on monocytes and macrophages (Hart and

Prickett, 1993). Costimulatory molecules such as CD80 (B7.1) and CD86 (B7.2), and molecules regulating costimulation such as CD40 are also expressed on mature myeloid DCs. DCs do not express surface differentiation antigens found on B cells (CD19, CD20), T cells (CD3), monocytes (CD14), and natural killer cells (CD56). Several antibodies have been described that preferentially but not exclusively stain mature DCs. Antibodies reactive against human DCs include anti-CD83 and CMRF-44 (Zhou et al, 1992; Hock et al, 1994). Antibodies directed at mouse DCs include 33D1, N418 (anti-CD11c), and DEC-205 (Kraal et al, 1986; Metlay et al, 1990).

DCs originate from the bone marrow and their precursors home via the bloodstream to almost all organs, where they can be found as sentinels in an immature state with high endocytic and phagocytic capacity. The current view is that these immature interstitial DCs are the precursors of the mature interdigitating DCs found in the T cell rich area of secondary lymphoid organs. Upon contact with bacterial DNA, LPS, dsRNA, and inflammatory cytokines such as TNF- or IL-1 the interstitial DCs change their phenotype and function and migrate to the germinal centers of regional lymph nodes, where they present antigens captured in the periphery to resting or naïve T cells and induce antigen-specific T cell responses.

With DC activation and migration from the tissue, antigen uptake activity and the associated antigen receptors are down regulated, resulting in a switch in APC function from antigen uptake to antigen presentation (Hart and McKenzie, 1988). DCs are capable of processing antigen via classical pathways: endogenous antigens via the proteasome into the MHC class I compartment, and exogenous antigens via endocytic lysosomes into the MHC class II compartment (Lanzavecchia, 1996). DCs also process alternative pathways of antigen processing and can route exogenous antigen into the MHC class I pathway through a mechanism known as cross-priming (Norbury et al, 1997). DCs may also utilize molecular chaperones such as heat shock proteins (hsp96) to deliver antigens via the class I pathway (Arnold-Schild et al, 1999).

The family of human DC displays considerable heterogeneity and plasticity at the level of phenotype and function (Banchereau et al, 2000). DC may be derived from two potential lineages: myeloid or lymphoid. Myeloid progenitors give rise to two main precursors: CD14⁺ CD11c⁺ precursors and CD14⁻ CD11c⁺ precursors (Caux et al, 1996). CD14⁺ CD11c⁺ cells differentiate in the presence of IL-4 and GM-CSF into interstitial DCs that correspond to dermal DCs *in vivo* (Grassi et al, 1998). In the presence of M-CSF, CD14⁺ CD11c⁺ precursor cells acquire characteristics of macrophages. CD11c⁺ DCs may be also reverted to macrophages under the same conditions. CD14⁻ CD11c⁺ precursors yield DC of the Langerhans cell type in response to GM-CSF, IL-4 and TGF-. Immature dermal or Langerhans type DC correspond to tissue resident sentinels in peripheral tissue sites. Upon encounter of T cell derived signals such as CD40L or microbial products such as LPS, they might be further driven along their differentiation pathway to mature DC. Mature DC resides in T cell areas of lymph

nodes and marginal zone of spleen with stable phenotype and function. A third major subset of DC are CD14⁻ CD11c⁻ IL-3R⁺ DC precursors (Grouard et al, 1997). These cells depend on IL-3 as survival factor and may be matured through CD40 signaling. Further surface markers include CD45RA and ILT-3 (Cella et al, 1999). They display low phagocytic activity and are the major source of IFN- γ production in response to viral infection (Siegal et al, 1999).

III. What is the role of dendritic cell for the induction of tumor immunity?

Effector mechanisms against endogenous tumors include both cellular and humoral immunity. The majority of experimental systems clearly demonstrate that tumor immunity is largely provided by CD4⁺ T lymphocytes (Hung et al, 1998; Dembic et al, 2000; Qin and Blankenstein, 2000), CD8⁺ T lymphocytes (Celluzzi et al, 1996; Jenne et al, 2000; Terheyden et al, 2000) or NK cells (Fernandez et al, 1999). T cells, and possibly also NK cells, however, require activation by APCs, and in this context DCs are pivotal for this process. To activate naïve T cells, DCs take up, process and present antigen by their MHC molecules (Banchereau and Steinman, 1998). In addition, T cell activation requires engagement of co-stimulatory receptors on the T cell, adequate types and concentrations of T cell activating cytokines and T cell attracting chemokines, and maintenance of the activation signal over a sufficient period of time. Currently, the list of family of co-stimulatory molecules is increasing dramatically, and it appears likely that DCs can minutely control the outcome of immune activation by means of differential surface receptor expression (Coyle and Gutierrez-Ramos, 2001), and that T cells in turn signal back to modulate the function of DCs (Bennett et al, 1998; Ridge et al, 1998; Schoenberger et al, 1998). Activation of NK cells and of macrophages is less well understood, but an interaction between DCs and these cell types has been demonstrated (Fernandez et al, 1999). Apart from generating a powerful antitumor immune response, DCs may also play an active role in the eradication of tumors themselves, since DCs have been shown to kill tumor cells via expression of death receptor ligands (Fanger et al, 1999), and recent data suggest that DCs activated by pro-inflammatory cytokines or LPS can directly inhibit the growth of tumor cell lines (Chapoval et al, 2000). Thus DCs are at the very center of a developing tumor-specific immune response, and are involved both in the initiation of tumor-specific immunity and the generation of immune effector functions.

Promising results obtained in a variety of murine tumor models using DCs presenting tumor antigens as well as the identification of a growing number of T cell epitopes presented by human malignant cells prompted the rationale for evaluating the efficacy of DC-based vaccines in clinical studies.

IV. Generation of dendritic cells for cancer immunotherapy

Clinical trials of DC vaccination have been made possible by the development of methods for obtaining large numbers of human DCs. Three general approaches have been exploited for use in clinical trials (**Table 1**). Myeloid DCs can be directly purified from blood by density-gradient centrifugation procedures (Hsu et al, 1996). The systemic administration of Flt-3 ligand or G-CSF increases blood DC numbers several fold (Maraskovsky et al, 2000; Pulendran et al, 2000). Alternatively, DCs can be prepared from CD14⁺ blood monocytes by *in vitro* culture with GM-CSF and IL-4 for 5-7 days, and differentiation with maturation stimuli (Bender et al, 1996; Romani et al, 1996). Maturation is essential to prevent reversion to monocytes. Autologous monocyte conditioned medium (MCM), CD40L or a cocktail of TNF- α , IL-1, IL-6 and PGE-2 are available for the maturation of DCs (Reddy et al, 1997; Thurner et al, 1999). CD34⁺ hematopoietic progenitor cells obtained from bone marrow, umbilical cord blood or peripheral blood following treatment with GM-CSF or G-CSF are also source of DC precursors. Following culture in GM-CSF and TNF- α (Caux et al, 1997), and stem cell factor or Flt-3 ligand, a mixed population of immature DCs with characteristics of both Langerhans cells and interstitial DCs has been obtained. Comparative studies will be required to establish differences between these various sources of DCs.

V. Critical parameters for optimal DC vaccination

Apart from choosing the right source of DC, critical issues for successful vaccination involve choice of antigen, antigen loading, route and schedule of administration, as well as immuno-monitoring.

The choice of DC is likely to depend on the type of antigen used. The cellular machinery required for processing antigen differs according to whether it is delivered as a peptide, protein or genetic vaccine. Immature DCs, which are actively endocytic and can internalize exogenous antigens efficiently, may be most suitable for the delivery of protein or complex antigens that require processing by the DC. In contrast, mature DCs, with higher expression of MHC molecules, may be more suitable for peptide-based protocols. Strategies to enhance DC function genetically to improve vaccine delivery and the subsequent induction of a powerful immune response are also being evaluated. These include genetic manipulation of DC to express cytokines or immuno-stimulatory molecules that can potentiate DC-T-cell interactions (Philip et al, 1998).

Wide range of antigenic preparations are available for loading of DC (**Table 2**). Peptide antigens are well defined antigenic epitopes binding to a defined set of MHC molecules, and easily accessible for immuno-monitoring of a peptide specific T cell response. However, peptide approaches are limited by the requirement of analysis of the MHC background of patients and the

Table 1. DC types available for clinical studies

Peripheral blood DC populations

Directly isolated from blood or expanded *in vivo* by growth factors, such as Flt-3 ligand or G-CSF

DCs derived from CD14⁺ monocytes

Monocytes enriched by anti-CD14 immunomagnetic beads or by plastic adherence

DCs derived from CD34⁺ hematopoietic progenitors

From bone marrow, peripheral blood or cord blood

knowledge of the sequence of the relevant peptide epitope. The use of whole protein antigens, DNA, RNA or recombinant viruses encoding the antigen of choice allows host HLA molecules to select the appropriate peptide epitope for presentation as peptide-MHC complex on the cell surface. This approach does not require analysis of MHC molecules, although we have to be aware of the fact that spectrum of epitopes seen by effector T cells might be restricted, since certain peptides are not presented by DC due to incomplete processing at the level of the proteasome (Morel et al, 2000). Recently, other approaches have been applied to use the entire antigenic content of a tumor cell for vaccination to present as many tumor antigens as possible to the immune system and minimize the occurrence of immune escape variants. This might be achieved by either pulsing DCs with whole tumor cell lysate (Ashley et al, 1997), tumor derived RNA (Nair et al, 1998), DNA (Philip et al, 1998) or fusion of tumor cells and DCs (Hart and Colaco, 1997). This technique does not require the definition of the TAA or MHC haplotype of the patients and has the potential for broad clinical application. The limitation of this approach is the availability of tissue serving as a source of tumor lysate or tumor derived RNA. A major disadvantage in using whole tumor in the form of lysates, RNA or DC-tumor fusions is that monitoring effector cells functions *in vitro* and *in vivo* is difficult to achieve.

After pulsing with tumor antigen, DCs need to be administered in an effective way to the cancer patients. Subcutaneous, intradermal, intravenous and intranodal approaches to deliver DCs have been evaluated clinically.

The intranodal approach (Nestle et al, 1998) bypasses the requirement for vaccine-loaded DCs to migrate to lymphoid tissue and simply relies on their capacity to express effective T cell stimulatory capacity. The intravenous route results in the accumulation of DCs to lung, liver, spleen and bone marrow, but not the lymph nodes or tumor sites (Morse et al, 1999). In contrast, studies using intradermal injection of monocyte derived DCs have demonstrated direct migration of DCs to the draining lymph nodes. However, these particular studies used immature DCs, and only ~1% of DCs migrated to the regional lymph node, the majority remained at the injection site (Thomas et al, 1999). In contrast, monocyte-derived DCs matured *in vitro* have shown impressive immune responses (Schuler-Thurner et al, 2000). It is unclear whether this effect was due to efficient migration and antigen-presentation induced by *in vitro* activation of DCs.

Detection of an antigen specific immune response is an important surrogate marker to control for an effective vaccination strategy even though correlation with clinical response will be the most important issue. The classic way to detect CTL activity is measurement of lytic activity against ⁵¹Cr labeled target cells. Since precursor frequencies are low, *in vitro* re-stimulations are often necessary in order to reach a CTL frequency detectable by cytotoxicity assays. Although these techniques are time consuming, still this method is the gold standard since it measures lytic activity of effector cells.

Table 2. Delivery of antigens by dendritic cells

Known tumor associated antigens:

- Synthetic or eluted peptides
- Recombinant or purified protein
- Non-peptide antigens, such as carbohydrates (e.g. MUC-1) or glycolipids (e.g. GM2)
- Transfection with cDNA or RNA encoding known tumor-associated antigen
- Recombinant viruses (adenoviruses, vaccinia, or retroviruses)

Approaches when antigens are unknown:

- Differentiation of DCs from malignant cells (acute myelogenous leukemia, chronic myeloid leukemia)
- Tumor-DC fusions
- DC-derived exosomes
- Tumor RNA
- Apoptotic or necrotic tumor cells
- Tumor lysates

These tests quantify antigen-specific T cells in the blood. It is unclear whether the blood is the best place to look for evidence of emerging immunity against vaccination, or whether the tumor site or draining lymph nodes may be more appropriate. Recently introduced methods rely on measurement of release of cytokines by CTL after contact with antigen (Romero et al, 1998). Cytokines may be measured by an ELISA method (ELISPOT) or quantified by intracellular cytokine staining and detection by flow cytometry. Detection of cytokine release does not necessarily correlate with the cytolytic activity of a given cell. Peptide-MHC tetrameric complex is another tool for detection of an antigen specific immune response.

However, this technique may not be able to detect low or intermediate affinity T cells, which are important in the context of vaccination against self-antigens. An additional method is delayed type hypersensitivity (DTH) testing for peptide specific immune responses. Peptide DTH testing was demonstrated in humans (Nestle et al, 1998) and this technique is easy to perform even though objective read out might be a problem and is observer dependent. DTH reactions are important since it measures induction of an antigen specific immune response. It is therefore crucial to develop appropriate immunological assays that may predict clinical responses more closely.

Table 3. Published clinical trials conducted with DC-based vaccines against cancer

Disease type	Antigen	DC type	Route	Investigator
Melanoma	a) Melan-A, gp100, tyrosinase	mDC	i.v.	Lotze MT et al (1997)
	b) MAGE-1, MAGE-3, Melan-A, gp100, tyrosinase	mDC	intranodal	Nestle FO et al (1998)
	c) MAGE-3	mDC	s.c., i.v.	Schuler-Thurner B et al (2000)
	d) MAGE-1, MAGE-3, Melan-A, gp100, tyrosinase	CD34-DC	i.v.	Mackensen A et al (2000)
	e) MART-1, gp100	mDC	i.v.	Panelli MC et al (2000)
	f) Melan-A, MAGE-3, gp100, tyrosinase	CD34-DC	s.c.	Banchereau J et al (2001)
	g) Tulys	mDC	i.d.	Chang AE et al (2002)
	h) tumor cell-DC fusion	mDC	s.c.	Krause SW et al (2002)
	i) Mart-1 ₂₇₋₃₅ peptide	mDC	i.v., or i.d.	Butterfield LH et al (2003)
	j) tumor cells	mDC	i.d.	O'Rourke MGE et al (2003)
	k) acid-eluted peptides	mDC	i.d.	Smithers M et al (2003)
Lymphoma	a) idiotype	PBDC	i.v., s.c. boost.	Hsu FJ et al (1996)
	b) idiotype	PBDC	i.v. s.c. boost	Timmerman JM et al (2002)
	c) Tulys	mDC	intranodal	Maier T et al (2003)
Myeloma	a) idiotype	mDC	i.v.	Lim SH et al (1999)
	b) idiotype	PBDC	i.v. s.c. boost	Reichardt VL et al (1999)
	c) idiotype	CD34-DC	s.c., s.c. boost	Titzer S et al (2000)

Prostate	a) PSMA	mDC	i.v.	Tjoa BA et al (1999)
	b) Fusion protein (PAP+GM-CSF)	PBDC	i.v., s.c.	Burch PA et al (2000)
	c) Fusion protein (PAP+GM-CSF)	PBDC	i.v.	Small EJ et al (2000)
	d) PSM-P1, PSM-P2	mDC	i.v.	Lodge PA et al (2000)
	e) rmPAP	PBDC	i.v., i.d., or intranodal	Fong L, Brockstedt D et al (2001)
RCC	a) Tulys	Allogeneic mDC	i.v.	Holtl L et al (1999)
	b) tumor cell-DC fusion	Allogeneic mDC	s.c., s.c. boost	Kugler A et al (2000)
	c) Tulys	mDC	i.d.	Oosterwijk-Wakka JC et al (2002)
	d) Tulys	mDC	s.c.	Marten A et al (2002)
	e) tumor cell-DC fusion	Allogeneic mDC	i.d.	Marten A et al (2003)
	f) tumor RNA	mDC	i.v., i.d.	Su Z et al (2003)
Liver	Tulys	mDC	intranodal	Iwashita Y et al (2003)
Various	Tulys	mDC	i.d.	Geiger J et al (2001)
Bladder	MAGE-3	mDC	s.c.	Nishiyama T et al (2001)
CEA-expressing malignant Lung, colon	CAP-1 (CEA peptide) 610D (CEA peptide)	mDC FL mobilized, density purified DC	i.v., i.d. i.v.	Morse MA et al (1999) Fong L et al (2001)
Stomach, esophagus, colon	MAGE-3	mDC	i.v.	Sadanaga N et al (2001)
Colon	CEA mRNA	mDC	i.v., i.d.	Morse MA et al (2003)

mDC, monocyte-derived DCs; CD34-DC, DCs derived from CD34-positive progenitors; PBDC, peripheral blood-derived DCs; i.v. intravenous; s.c., subcutaneous; i.d., intradermal; MAGE, melanoma antigen; PSMA, prostate-specific membrane antigen; PAP, prostatic acid phosphatase; PSM-P, prostate-specific membrane antigen-derived peptide; rm, recombinant mouse; RCC, renal-cell carcinoma; Tulys, tumor lysate; FL, Flt-3 ligand.

VI. Dendritic cell-based vaccines in clinical trials

Numerous trials are currently ongoing or planned in the very fast moving field of DC immunotherapy trials. We will only discuss published trials in DC vaccination that includes malignant melanoma, non-hodgkin lymphoma, multiple myeloma, prostate cancer, renal-cell carcinoma, liver cancer, pediatric solid tumor, bladder cancer and colorectal cancer (**Table 3**).

A. Melanoma

Many immunologically relevant melanoma antigens including differentiation antigens Melan-A, gp100, and tyrosinase, as well as cancer-testis antigens, such as those of the melanoma antigen (MAGE) family are currently

being investigated in DC-based clinical studies.

Lotze and co-workers (Lotze et al, 1997; Lotze et al, 1998) used monocyte-derived DCs to treat HLA-A2 positive patients with metastatic melanoma. DCs were pulsed with peptides derived from Melan-A, gp100, or tyrosinase. Twenty-eight patients received weekly intravenous and subcutaneous infusions of peptide-pulsed DCs for four weeks. Two patients achieved a complete response and 1 patient responded partially, although, one of the complete responders later developed overt rheumatoid arthritis.

Nestle and colleagues (Nestle et al, 1998; Nestle, 2000) used monocyte-derived DCs pulsed with MAGE-1 and MAGE-3 peptides (for patients expressing HLA-A1), Melan-A, gp100 and tyrosinase peptides (for patients expressing HLA-A2), or MAGE-3 and tyrosinase peptides

(for patients expressing HLA-B44). Patients with metastatic melanoma received weekly intranodal vaccinations for four weeks with a fifth injection in week 6 and then patients had monthly injections for up to 10 months, depending on clinical response. Vaccinations were well tolerated and 8 of 30 patients had clinical responses, with 3 complete and 5 partial remissions.

Thurner and colleagues (Thurner et al, 1999) used monocyte-derived DCs pulsed with MAGE-3 peptides to treat HLA-A1 positive patients with metastatic melanoma. Five vaccinations (three subcutaneous followed by two intravenous) were given on every 2 weeks. Six of 11 patients had mixed responses. Eight patients showed an increase in MAGE-3-specific CTL responses. A follow-up study (Schuler-Thurner et al, 2000) involving 12 patients with stage IV melanoma used the same procedure as described by Thurner and colleagues. Three to five vaccinations with mature, monocyte derived DCs loaded with HLA-A2 restricted peptides for MAGE-3 and for influenza matrix generated vigorous immune responses as detected by *in vitro* assays in all eight vaccinated patients. However, no significant clinical responses were observed after final vaccination. Four patients died early in the treatment period due to disease progression. Only 1 patient had stable disease, but disease progressed in all remaining patients.

Mackensen and colleagues (Mackensen et al, 2000) did perform a phase I study in 14 patients with advanced melanoma using CD34 derived DCs matured with TNF- α . DCs were pulsed with MAGE-1 and MAGE-3 peptides (for patients expressing HLA-A1) or Melan-A, gp100 and tyrosinase peptides (for patients expressing HLA-A2). Patients received at least four intravenous vaccinations, biweekly. Patients with stable or responding disease continued to receive vaccination every four weeks until disease progression. The vaccines were tolerated. One patient had a mixed response and six patients had stable disease for 3 to 8 months. Peptide-specific DTH response was observed in 4 patients and expansion of peptide specific CTL response was observed in 1 patient. A similar study was conducted (Banchereau et al, 2001) in 18 HLA-A 0201⁺ patients with stage IV melanoma using CD34 derived DCs. Patients were immunized with DCs pulsed with peptides derived from four melanoma antigens (MelAgs) Melan-A/MART-1, tyrosinase, MAGE-3, and gp 100 subcutaneously every two weeks for a total of four vaccinations. DC injections were well tolerated except for two patients. DC vaccination resulted enhanced immunity to 1 MelAgs in 16 of 18 patients. Ten of 14 evaluated patients developed DTH to at least one peptide after repeated DC vaccination. The development of T cell response to multiple tumor antigens on peptide-pulsed DCs in this study was associated with a favorable early clinical outcome. Seven of 17 evaluable patients experienced tumor progression. The remaining 10 patients did not progress at this time point (10 weeks from study entry). Among these, four patients had regression of tumor metastases at one or more disease sites and three patients cleared any evidence of disease.

Recently, Butterfield and colleagues (Butterfield et al, 2003) have conducted a phase I study in 18 HLA-

A 0201⁺ patients with stage III-IV melanoma using monocyte derived DCs pulsed with MART-1₂₇₋₃₅ epitope. Patients received three intravenous or intradermal injections at intervals of two weeks. The results of this trial have shown that immunizations with DCs pulsed with single MHC class I peptide could generate robust peptide-specific T cell responses in most patients without toxicity. The investigators have suggested that intradermal route of immunization resulted stronger MART-1-specific immunity compared with intravenous route. An expansion of MART-1₂₇₋₃₅-specific T cells was observed in most patients by MHC class I tetramer and IFN- γ ELISPOT analysis. After completion of vaccination schedule, out of ten patients with stage IV melanoma, one patient had complete response and two patients had disease stabilization. Five patients with stage III disease have had no evidence of relapse at a median of 24+ months of follow-up.

Another study was conducted in 19 patients suffering from stage IV melanoma (O'Rourke et al, 2003). DCs were generated from monocytes and cultured with irradiated autologous tumor cells. Patients received tumor antigen-loaded DCs by intradermal infusion, two weeks apart and boosted five times. Treatment was well tolerated and DCs vaccination resulted autologous tumor cells specific DTH response in 3 out of 10 patients. Three of 12 patients, who completed the immunization schedule, had complete responses, three had partial responses and remaining six had progressive disease.

Smithers and co-workers (Smithers et al, 2003) performed a phase I/II study in 22 patients with metastatic melanoma by immunization with autologous melanoma peptides and particulate hepatitis B antigen (HBsAg)-exposed immature monocyte derived DCs. Patients received 8 intradermal DC vaccinations, each two weeks apart. No major toxic effects were observed during vaccination. Nine out of 19 patients receiving HBsAg-exposed DCs, primed or boosted a cellular immune response to HBsAg. Four out of 9 HBsAg-responding patients achieved objective melanoma specific clinical responses (one complete and two partial responses) and one patient's disease stabilized for 2 months. None of the 3 patients receiving DCs exposed only to melanoma peptides responded clinically.

Malignant melanoma has been shown to be susceptible to T cell-mediated immunity and, therefore, is a candidate for vaccination approaches. Recent clinical trials with DCs as a cellular adjuvant have concentrated on defined peptides as the source of antigens, and rely on foreign proteins as a source of help to generate a cell-mediated immune response. Initial data provide encouragement for targeting melanoma Ags in the clinic, however, additional studies are needed to establish and optimize their clinical efficacy.

B. Lymphoma

In the first reported DC trial (Hsu et al, 1996), the effect of autologous DCs pulsed *ex vivo* with tumor-specific antigen was investigated in 4 patients with malignant B cell lymphoma who had failed conventional chemotherapy. After leukapheresis, peripheral blood DCs

were subjected to a sequence of enrichment steps, before being pulsed with idiotypic protein and infused intravenously. Patients received three immunizations of idiotypic protein-pulsed DCs at intervals of four weeks. Patients also received subcutaneous injections of idiotypic protein or KLH in saline two weeks after each vaccination to boost the primary response induced by the DCs infusion. A fourth idiotypic protein-DC immunization was given 5-6 months after study entry. Treatments were well tolerated, and induction of idiotypic protein specific immune response was observed as well as complete clinical regression in 2 out of 4 patients. One patient remained in complete remission for more than three years (Fong and Engleman, 2000). A recent follow-up study (Timmerman et al, 2002) involving 35 patients with stage III or IV B-cell non-Hodgkin lymphoma (NHL) confirmed idiotypic-specific immune responses and durable clinical responses. Therapy consisted of significant clinical outcome as 4 patients had complete responses, 2 patients had partial responses, and 1 patient had molecular response. In evaluable patients with residual tumor at the time of vaccination, tumor regression was observed in 4 of 18 patients and 16 of 23 patients remained progression free at a median of 43 months after the completion of chemotherapy.

Another study (Maier et al, 2003) was conducted in 10 patients with cutaneous T cell lymphoma. Patients received intranodal immunization of mature monocyte-derived DCs pulsed with autologous tumor lysate once a week for 8 weeks. Immunizations were tolerated with minor adverse effects and 5 patients had objective responses (one complete and four partial responses). Clinical response was associated with tumor-specific DTH response, tumor lysate specific proliferative response, and increased IFN- γ production.

In NHL, therapeutic options are limited, especially in advanced stages. DCs vaccination was feasible, well tolerated, and induced immunologic and clinical responses in patients with B cell or T cell lymphoma. Clinical studies conducted with B cell lymphoma patients were promising, as DCs vaccination was able to induce both humoral and cellular immune responses and regression of tumor burdens with prolonged survival.

C. Myeloma

Lim and Bailey-Wood (Lim and Bailey-Wood, 1999) have reported a study of idiotypic protein pulsed DC vaccination in myeloma. Six patients were treated with monocyte-derived DCs pulsed with idiotypic protein. Patients received three intravenous DC vaccinations, each two weeks apart. The immunizations were well tolerated. The majority of patients showed evidence of increased immune response toward idiotypic protein. One patient showed a 25% fall in serum idiotypic protein that was sustained over 13 months, and in two other patients it remained constant 8 months after the completion of vaccination.

Reichardt and colleagues (Reichardt et al, 1999) conducted a feasibility study for DC vaccination after autologous peripheral-blood stem-cell transplantation for patients with myeloma. After 3-6 months of

transplantation, these patients were immunized with idiotypic protein pulsed-DCs intravenously and the immunization was repeated after four weeks. Patients were boosted five times with idiotypic protein-KLH on every four weeks from four weeks after the second idiotypic protein-DC vaccine. Two out of 12 patients developed an idiotypic-specific cellular proliferative immune response and 1 of 3 patients studied developed a transient but idiotypic-specific CTL response.

In a study by Titzer and co-workers (Titzer et al, 2000), 11 patients were enrolled, 10 with stage III disease and 1 with stage II disease. Patients were immunized with idiotypic protein pulsed DCs subcutaneously followed by three booster immunizations, given every other week with a combination of idiotypic protein and GM-CSF (nine patients) or with idiotypic-derived peptide pulsed DCs (two patients). After vaccination, clinical benefit was observed in 1 patient with a fall in bone-marrow plasma-cell infiltrate. Three of 10 patients studied had increased amount of antibody to anti-idiotypic protein. An increased T cell response specific for idiotypic protein was also observed in 4 patients. The results of these studies suggest that treatment with idiotypic protein-pulsed DCs is safe, and can result in disease stabilization and possibly also in objective clinical responses.

D. Prostate cancer

Murphy and Tjoa have studied extensively on the use of peptide-pulsed DCs for patients with locally advanced or metastatic prostate cancer (Tjoa et al, 1997; Tjoa et al, 1998; Tjoa et al, 1999; Murphy et al, 1999). Monocyte-derived DCs were pulsed with peptides derived from prostate-specific membrane antigen (PSMA) and were administered intravenously every 6 weeks for a total of six infusions. In a phase II trial, 33 patients were enrolled (Murphy et al, 38, 1999) and of 25 evaluable patients, 8 patients responded (two complete and six partial responses) as identified by declining levels of serum marker. In a group of 37 patients with recurrent disease after primary therapy (Murphy et al, 39, 1999), 11 patients responded (one complete and ten partial responses).

Another study was conducted in 13 patients suffering from progressive hormone-refractory metastatic prostate carcinoma (Burch et al, 2000). DCs were generated from circulating blood precursors and pulsed with fusion protein (PA2024) consisting of human GM-CSF and human prostatic acid phosphatase (PAP). Patients received two doses of antigen-loaded DCs by intravenous infusion, one month apart, followed by three subcutaneous doses of PA2024 with monthly intervals. Circulating prostate-specific antigen levels decreased in three patients and antigen specific T cell responses were detected in most of the patients within four weeks of the first DC infusion.

Small and colleagues (Small et al, 2000) also used PA2024-loaded DCs in patients with hormone-refractory prostate cancer. Twelve patients were enrolled in the phase I trial and 19 in the phase II trial. DCs were generated from circulating blood precursors and pulsed with fusion protein PA2024. Patients received DC-vaccine, intravenously, in week 0, 4, and 8. A fourth infusion was

administered in week 24, to patients with stable disease. Six patients in the phase I study also received DCs loaded with KLH. The treatment was well tolerated. All patients developed antigen specific T cell proliferative responses after second or third vaccination. Circulating prostate specific antigen levels decreased at least 50% in 3 patients and 25-49% in another 3 patients. For patients in the phase II study, median time to disease progression was 29 weeks. The investigators suggested that response was correlated with the development of an immune response to PAP and dose of DCs used.

To explore the potential role of xenoantigen immunization in cancer patients, Fong and colleagues (Fong, Brockstedt et al, 2001) performed a phase I clinical trial in 21 patients with metastatic prostate cancer using DCs pulsed with recombinant mouse PAP as a tumor vaccine. Patients were immunized twice with PAP-loaded DCs 4 weeks apart via intravenous, intradermal, or intralymphatic injections. Patients tolerated the vaccinations without significant toxicity. All patients developed T cell response to mouse PAP and 11 of 21 patients also developed T cell response to the homologous self-Ag. Th1 associated immune response was observed with secretion of IFN- and/or TNF- . Six of 21 patients had evidence of disease stabilization following completion of the vaccinations as determined by serum prostate specific antigen levels and radiographic imaging. However, no correlation was observed between clinical stabilization, and route of DC administration or the development of anti-PAP antibodies.

These results suggest that clinical trials with DC-based vaccines are promising with positive clinical outcome. Protein-pulsed DCs are likely to be more effective vaccine candidate as it can stimulate both CD4⁺ and CD8⁺ T cells, whereas peptide-pulsed DCs are designed to stimulate CD8⁺ T cells only. The studies involving recombinant tumor-antigen fusion protein were also remarkable. The use of whole protein was advantageous over peptides in that the eligibility need not be restricted to particular HLA types, since patient's own DCs would process the protein and present it in the context of the patient's own HLA type.

E. Renal-cell carcinoma

Holtl and co-workers (Holtl et al, 1998; Holtl et al, 1999) used monocyte-derived DCs to treat patients with metastatic renal-cell carcinoma. DCs were pulsed with tumor-cell lysate and KLH. Patients received three intravenous vaccinations of antigen-pulsed DCs at one month intervals. One patient had a partial response and two others had stable disease.

Kugler and colleagues (Kugler et al, 2000) used a different DC approach in the treatment of metastatic renal-cell carcinoma. They developed tumor-cell-DC hybrids by electrofusion. Autologous tumor cells were fused with allogeneic monocyte-derived DCs and irradiated before vaccination. Seventeen patients were vaccinated subcutaneously and received a booster injection after 6 weeks. Patients with stable disease received continuous booster immunizations every 3 months. Treatment was well tolerated and 7 patients responded (Four complete,

two partial, and one mixed responses) to this therapeutic strategy. Marten and colleagues (Marten et al, 2003) also used monocyte-derived DCs fused with either allogeneic or autologous tumor cells for vaccination of patients with progressive metastatic renal-cell carcinoma. Twelve patients were treated with tumor-cell-DC hybrids intradermally every 4 weeks for a total of three infusions. The vaccine was well tolerated in all patients with no toxicity. After completion of vaccinations, four patients remained in a stable disease state with positive responses to DTH and *in vitro* cytotoxicity. Interestingly, there was no difference in clinical outcome between vaccination with allogeneic and autologous tumor fusions.

Recently, Oosterwijk-Wakka and colleagues (Oosterwijk-Wakka et al, 2002) have conducted a phase I study in twelve patients with metastatic renal cell carcinoma. Patients received intradermal immunizations with autologous immature DCs pulsed with autologous tumor lysate, three times, each two weeks apart. The treatment was combined with low-dose of IL-2. IL-2 was administered for five consecutive days after each vaccination. In six patients, KLH was added to the DCs culture to monitor immunologic response. After vaccinations, cellular anti-KLH response was observed, however, tumor lysate specific proliferative response and humoral responses specific for tumor lysate or KLH were absent. This vaccination strategy with immature DCs had little benefit for patients with advanced renal-cell carcinoma as no objective clinical response was observed at the end of the study. Results from a different clinical trial in which patients with metastatic renal cell cancer were treated with autologous tumor lysate pulsed DCs showed moderate immunologic responses, with an increase in cytotoxic activity of PBLs against renal cell carcinoma cells and an increase in CD3⁺ CD28⁺ cells. In this trial seven patients remained with progressive disease, seven patients showed stable disease after treatment, and one patient developed a partial response (Marten et al, 2002).

Metastatic renal cell carcinoma (RCC) remains a therapeutic challenge because of its demonstrated resistance to conventional means of therapy. Although the administration of recombinant cytokines has become an accepted standard treatment for patients with metastatic RCC, overall response rates have remained unsatisfactory. Preliminary results using DCs in RCC demonstrate that this treatment modality is well tolerated and can be associated with strong immunological responses. These data indicate a potential role of DCs vaccines for the induction of active immunity in patients with advanced RCC. In addition, observed immunologic changes in patients suggest an activation of the anti-tumoral immune response.

F. Liver cancer

In a recent study by Iwashita and co-workers (Iwashita et al, 2003), 10 patients were enrolled with unresectable primary liver cancer. Monocyte-derived DCs were pulsed with tumor lysate and KLH. Patients received four vaccinations at weekly intervals. Immunization was continued at monthly intervals for up to 12 vaccinations

depending on clinical response. DCs were administered into inguinal lymph nodes under ultrasound control. Treatment was well tolerated and DCs vaccination resulted KLH specific DTH response in 7 out of 10 patients. Three patients responded as serum levels of tumor markers decreased after vaccination in two patients and in one patient, one of the two liver tumors decreased in size and showed necrotic change after eight vaccinations. These results indicate that immunization by tumor lysate pulsed DCs were feasible in unresectable primary liver cancer patients without measured toxicity.

G. Pediatric solid tumor

Geiger and colleagues (Geiger et al, 2001) reported the results of a tumor lysate-pulsed DCs vaccine approach in a phase I trial of pediatric patients with solid tumors. Patients with neuroblastoma, sarcoma, and renal-cell carcinoma were treated in this study. DCs were generated from monocytes, after leukapheresis. Monocyte-derived DCs were pulsed separately with tumor cell lysates and KLH and then combined. Patients received three vaccinations, every 2 weeks, by intradermal injection near the inguinal lymph nodes. Fifteen patients were enrolled. Of 10 evaluable patients, DTH response was detected in 7 patients for KLH and 3 of 6 patients for tumor lysates. T cell priming specific for KLH was observed in 6 of 10 patients and to tumor in 3 of 7 patients as demonstrated by specific IFN- secreting T cells. Five patients showed stable disease, including 3 who had minimal disease during vaccinations and remained tumor free for 16-30 months. Significant regression of multiple metastatic nodules was also observed in one patient with fibrosarcoma. The present clinical trial suggested that DCs vaccination approach is feasible in children with solid tumors. Because of its low toxicity and ability to generate specific immune responses, the use of DC-based tumor vaccines in children may become more beneficial in minimum disease setting.

H. Bladder cancer

Nishiyama and co-workers (Nishiyama et al, 2001) have reported a pilot study of MAGE-3 peptide pulsed DC vaccination in four patients with advanced bladder cancer expressing MAGE-3 gene. Monocyte-derived DCs were generated after leukapheresis. DCs were pulsed with MAGE-3 epitope peptide (IMPKAGLLI) and patients received at least 6 subcutaneous DC vaccinations, each two weeks apart. The treatments were continued at regular intervals for up to 18 vaccinations depending on clinical response. During vaccinations, no significant side effects were noted in these patients. Three of four patients responded (one complete and two partial responses) with significant reductions in the size of lymph node metastasis and/or liver metastasis. They have also reported induction of MHC class I-restricted MAGE-3 specific CTLs *in vitro* in MAGE-3+ bladder cancer. Melanoma antigens such as the MAGE family are now recognized as tumor-rejection antigens and are expressed in various tumors, including bladder cancers and melanoma, but not in normal tissues. The present study indicates that MAGE-specific cancer immunotherapy might be one of the more attractive and

effective strategies for the treatment of advanced bladder cancer.

VII. Dendritic cell-based vaccines directed against colorectal cancer

Carcinoembryonic antigen (CEA) is a 180-kDa membrane intercellular adhesion glycoprotein that is over expressed by a significant proportion of human tumors including >90% of colorectal, gastric, and pancreatic cancers, 70% of nonsmall cell lung cancer, and 50% of breast cancer. Human trials using DCs loaded with CEA in the form of peptide or mRNA are underway at several institutions. In the first reported DC trial (Morse et al, 1999), the effect of autologous monocytes-derived DCs pulsed with the HLA-A2-restricted CEA peptide CAP-1 was investigated in patients with advanced CEA-expressing malignancies. In this phase I study, the first 12 patients received four DC vaccinations intravenously, a week apart. The last 9 patients received DCs intravenously, together with peptide-pulsed DCs intradermally every 2 weeks for four immunizations. The last patient received IL-2 subcutaneously each day for 4 days after each DC injection. There were no treatment related toxic effects. Of 19 evaluable patients, 1 patient had a minor response and another had stable disease. Two patients were found to have a peptide-specific DTH response after vaccination.

Fong and colleagues (Fong et al, 2001) performed a phase I study in 12 patients with metastatic colorectal or nonsmall cell lung carcinoma by immunization with a CEA-derived peptide. Flt-3 ligand, a hematopoietic growth factor, which expands DCs *in vivo*, was administered into patients subcutaneously for ten consecutive days before leukapheresis. The enriched DCs were pulsed *in vitro* with 610D peptide, an HLA-A*0201-restricted epitope from CEA (CEA₆₀₅₋₆₁₃) where aspartate was substituted for asparagine at position 610. Patients received immunizations with peptide pulsed DCs in one month apart by intravenous injection. No major toxic effects were observed during and after vaccination. They reported clinical responses in 5 of 12 patients. Two patients experienced tumor regression, 1 patient had a mixed response, and 2 exhibited stable disease for 3 to 6 months. Seven patients developed CTL response after vaccination. Clinical response correlated with expansion of CD8 tetramer⁺ T cells. Five patients had >1% tetramer⁺ CD8 cells and 6 patients had 0.5% tetramer⁺ CD8 cells after vaccination.

Sadanaga and colleagues (Sadanaga et al, 2001) have also reported a study of MAGE-3 peptide pulsed DC vaccination in 12 patients with advanced gastrointestinal carcinoma (six stomach, three esophagus, and three colon) expressing MAGE-3 gene. Monocyte-derived DCs were generated after leukapheresis. DCs were pulsed with peptide on day 7 of culture, and patients received four intravenous DC vaccinations, each 3 weeks apart. Treatment was well tolerated and clinical response was promising. In 7 patients, tumor markers decreased after the first or second vaccination compared with pre treatment level. Tumor regression evidenced by imaging studies was

observed in 3 patients. Peptide-specific immune response evidenced by DTH reactions, were observed in 3 patients after the fourth vaccination. This study has shown peptide-specific CTL responses in 4 of 8 patients after vaccination. Intracellular cytokine analysis was also performed for 6 patients. In 3 of 6 patients, the ratio of IFN- γ /IL-4 of CD4-positive cells increased after vaccination and 2 of these 3 patients had tumor regression. These results are interesting and may have favorable outcome in future clinical trial.

Morse and co-workers (Morse et al, 2003) have conducted a phase I/II study of CEA mRNA-pulsed DCs for patients with metastatic malignancies expressing this tumor antigen. In the dose-escalating phase I study 29 patients were enrolled and 13 patients in the phase II study. Monocyte derived DCs were pulsed with CEA mRNA and then administered intravenously and intradermally every 2 weeks for a total of four infusions. Eight patients also received IL-2 subcutaneously each day for four days after each DCs infusion. The immunizations were well tolerated. CEA-specific DTH response was observed in 3 of the 19 patients and 6 of the 13 patients in the phase I, and phase II study respectively. CEA-specific CTL response evaluated in 3 patients was moderate after immunization. Of the 24 patients evaluable for response in the phase I study, 6 patients achieved objective clinical responses or disease stabilization- 1 complete, 2 partial responses, and 3 cases of stable disease. In the phase II study, 9 of 13 patients have relapsed at a median of 122 days.

Another approach for the development of both cellular and humoral immune responses to known TAAs is the use of anti-idiotypic antibodies. Based on the immune network hypothesis of Lindemann (1973) and Jerne (1974), any epitope could be converted into idiotypic determinants expressed on antibodies. Anti-idiotypic (Id) antibodies that share sequence homologies with nominal TAA could act as functional mimics of T cell antigens and stimulate cellular immune responses. The authors have developed and characterized a murine anti-Id mAb, designated 3H1 or CeaVac. 3H1 was generated against an anti-CEA antibody, designated 8019, which reacts with a specific epitope on CEA that is highly restricted to tumor cells and not found on normal tissues (Bhattacharya-Chatterjee et al, 1990). 3H1 anti-Id antibody functioned as an internal image of CEA by generating anti-anti-Id (Ab3) responses in mice, rabbits and monkeys that recognized CEA and had a major antitumor effect in a murine tumor model (Pervin et al, 1997). In a phase I clinical trial, among 23 patients with advanced colorectal cancer, 17 generated anti-anti-Id Ab3 responses, and 13 of these responses were proven to be true anti-CEA responses (Ab1) (Foon et al, 1995; Foon et al, 1997). These patients were treated with aluminum hydroxide-precipitated 3H1. CeaVac have also shown promise as a potential vaccine candidate in phase II clinical trials (Foon et al, 1999) for colorectal cancer patients. Cellular immune responses observed in these patients were CD4⁺ Th1 type T cell responses.

One area of interest to us has been the development of new immune adjuvants that may augment the potency of 3H1 as a tumor vaccine. In our recent study (Saha et al,

2003), in a murine model of colon cancer, we have shown that efficacy of 3H1 could be improved by using bone marrow derived DCs as direct antigen presenting cells. In this study, immunization of naïve C57BL/6 (H-2^b) mice with 3H1-pulsed DCs induced humoral and cellular anti-3H1 as well as anti-CEA immunity. 3H1-DC immunization activated both MHC class II-restricted CD4⁺ T cells, as well as MHC class I-restricted CD8⁺ T cells. Mice immunized with 3H1 were protected against murine colorectal cancer cell line MC-38 transfected with human CEA (clone C15-4.3), whereas no protection was observed when 3H1 vaccinated mice were challenged with nontransfected parental MC-38 cells. One hundred percent of experimental mice immunized with 3H1-DC rejected CEA expressing C-15 tumor cells. The tumor rejection in 3H1-pulsed DC-treated mice was associated with the induction of a memory response that helped those mice to survive a second challenge with a lethal dose of C-15 cells. Currently, we are engaged to evaluate the potency of this vaccine in mice transgenic for human CEA. Our preliminary data in transgenic mice system appear promising and the implication of these findings for the use of 3H1-pulsed DCs as vaccine for CEA-positive human cancer patients need to be investigated.

These pre-clinical and clinical results provide evidence of safety and feasibility and induction of antigen-specific immunity using DCs based approach to immunotherapy. These studies also provide supporting evidence that the treatment of gastrointestinal cancers with antigen-pulsed DCs has a promising future. Given these promising results, additional clinical investigations are warranted to confirm and further characterize the efficacy of different approaches.

VIII. Opportunities and challenges for DC-based immunotherapy

Several published clinical studies have shown that generation of antigen-loaded DCs *ex vivo* on a clinical scale is possible and DCs vaccination is safe and well tolerated. Most of these phase I clinical trials have shown specific antitumor immune responses with some clinically meaningful outcome. However, the use of potent immunostimulatory platforms such as DCs carries a risk of inducing autoimmune responses against self-antigens, specifically if the target antigen is also expressed by normal cells (Ludewig et al, 2000). Increasing evidence suggest that DCs could prime T cell mediated autoimmune diseases (Pettit and Thomas, 1999; Drakesmith et al, 2000). Such DC-mediated autoimmunity could be due to cytokine-mediated dysregulation of DCs as well as defects in the expression of genes regulating DC functions (Drakesmith et al, 2000). Thus, the future of DC-based cancer vaccines will also depend on our capacity to evaluate and control the risk of eliciting such autoimmune responses.

Another important challenge, specifically when autologous DCs are used to prepare the vaccine, is that cancer patients are known to frequently exhibit defect in DC-based functions. This might include defect in antigen presentation, and in some cases in DC maturation

(Gabrilovich et al, 1996; Gabrilovich et al, 1997; Almand et al, 2000). Tumors themselves can secrete various mediators including IL-6, IL-10, VEGF that have recently been found to inhibit DC differentiation and/or maturation. The *in vitro* treatment of DCs must be optimized to resolve this issue. Alternatively, immunopotentiating molecules such as IL-2 (Shimizu et al, 1999) can be administered with antigen-loaded DCs to overcome the problem.

It remains questionable whether an immune reaction alone will be able to eradicate large tumor masses in advanced-stage disease. Human tumors are too heterogeneous in terms of the antigens they express and their susceptibility to immune-mediated killing. Immunotherapy in conjunction with standard treatments is likely to be more successful. Therefore more attractive option for tumor immunotherapy might be to maintain or resolve minimal residual disease, once the mass of tumor has been resected by conventional methods (Bodey et al, 2000). Future studies will improve this approach for modulating immunity in the clinic.

IX. Concluding remarks

Current therapeutic approaches to metastatic cancer, including chemotherapy and radiotherapy, have had little impact on survival for patients with a range of malignant diseases. It is obvious that we need to find out a better approach for more effective treatments. Recent advances in the field of tumor immunology have expanded our understanding of the nature of TAAs and mechanisms of T cell activation. Successful activation of T cell response to these TAAs requires that they be presented in the context of the appropriate co-stimulatory signals. Recent insights into the role of DCs as the potent antigen-presenting cells that initiate immune responses may provide the basis for generating more effective antitumor immune responses. Several forms of DC-mediated immunotherapy are currently being investigated with great intensity, using a wide variety of different vaccination protocols. Indeed, both preclinical as well as initial clinical results are very promising and clearly justify pursuing this approach. However, it is necessary to perform clinical studies in a well designed and well controlled fashion, since otherwise, negative clinical results will appear that may threaten the acceptance of the whole concept of DC-mediated tumor immunotherapy. Hopefully, our recent understanding of tumor immunology and current clinical approaches of DC-based vaccines will provide us help to develop more effective vaccines against cancer including colorectal cancer in near future.

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