[Frontiers in Bioscience 12, 4050-4060, May 1, 2007]

Dendritic cell vaccines

Paul J. Mosca [1,2] Amy C. Hobeika [1,2] Timothy M. Clay [1,2] Michael A. Morse [1,3], H. Kim Lyerly [1,2,4,5]

[1] Duke Comprehensive Cancer Center and Departments of [2] Surgery, [3] Medicine, [4] Pathology, and [5] Immunology, Duke University Medical Center, Durham, NC 27710

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Preparation of dendritic cells
- *3.1. Sources of dendritic cells*
- *3.2. Dendritic cell mobilization strategies*
- *3.3. Dendritic cell culture conditions*
- *3.4. Methods of loading dendritic cells with tumor antigens*
- *3.5. Dendritic cell maturation*
- *3.6. The role of helper antigens*
- *3.7. cryopreservation of dendritic cells*

4. Administration of dendritic cell vaccines
- *4.1. Route of dendritic cell administration*
- *4.2. Immunization schedule*
- *4.3. Use of vaccine Adjuvants*
- *4.4. Prime-boost strategy*

5. Clinical trials evaluating dendritic cell based cancer vaccines
- *5.1. Malignant melanoma*
- *5.2. Hematologic malignancies*
- *5.3. Genitourinary tract malignancies*
- *5.4. Gastrointestinal malignancies*
- *5.5. Other malignancies*

6. Perspective
7. Acknowledgments
8. Reference

1. ABSTRACT

Dendritic cells are antigen-presenting cells that have been shown to stimulate tumor antigen-specific T cell responses in preclinical studies. Consequently, there has been intense interest in developing dendritic cell based cancer vaccines. A variety of methods for generating dendritic cells, loading them with tumor antigens, and administering them to patients have been described. In recent years, a number of early phase clinical trials have been performed and have demonstrated the safety and feasibility of dendritic cell immunotherapies. A number of these trials have generated valuable preliminary data regarding the clinical and immunologic response to DC-based immunotherapy. The emphasis of dendritic cell immunotherapy research is increasingly shifting toward the development of strategies to increase the potency of dendritic cell vaccine preparations.

2. INTRODUCTION

Dendritic cells (DC) are specialized antigen-presenting cells that express high levels of major histocompatibility complex (MHC) and costimulatory molecules, which facilitate antigen presentation. Because the immune system in cancer patients may have inadequate or dysfunctional antigen presentation (1,2), the administration of DC-based cancer vaccines has received considerable interest in recent years. Preclinical investigations have indicated that DC loaded by a variety of strategies can induce tumor antigen-specific immune responses and have laid the groundwork for a number of recent phase I and II clinical trials (3). The purpose of this article is to discuss the principles of DC-based cancer vaccines and highlight some recent clinical trials in which they have been evaluated.

3. PREPARATION OF DENDRITIC CELLS

3.1. Sources of Dendritic Cells

Because diverse types of DC can be generated *in vivo* and *in vitro*, identifying the ideal source of DC for use in DC based cancer vaccines has been challenging (4,5). DC prepared by different methods may exhibit different properties, such as the ability to stimulate T-helper 1 (Th1) responses or Th2 responses (6-8).

DC comprise less than 1% of mononuclear cells in the peripheral blood (9). Leukapheresis can be used to isolate approximately 10^6 to 10^7 DC and may be adapted with positive or negative selection techniques (10-12). While the direct isolation of DC from peripheral blood allows rapid preparation of DC, it may require repeated leukapheresis if multiple immunizations are required in a protocol. This can present a significant problem, particularly for those cancer patients with functionally defective or decreased numbers of DC (13,14).

An alternative method of preparing DC is to generate them *ex vivo*. Most commonly, DC are cultivated from peripheral blood monocytes or CD34+ hematopoietic precursors. DC precursors may be enriched from peripheral blood mononuclear cells by techniques such as plastic adherence, density gradient centrifugation, positive selection of CD14+ cells, negative selection of B and T cells, and/or elutriation. DC may be cultivated by treating enriched precursor cells for approximately seven days with granulocyte macrophage-colony stimulating factor (GM-CSF) plus interleukin-4 (IL-4) (15), or with IL-13 (16-18). An advantage of this strategy is that more than 10^9 DC may be prepared from a single leukapheresis product (17). DC with potent antigen-presenting capacity may also be generated from CD34+ precursors (19-21).

Another innovative strategy for generating DC is to derive them from hematologic malignancies such as chronic myelogenous leukemia (CML) or acute leukemia (22-26). In CML, for example, a t(9:22) chromosomal translocation yields a gene encoding the fusion protein BCR-ABL, which contains T cell epitopes. CD34+ mononuclear cells isolated from patients with CML can be cultivated to generate DC bearing BCR-ABL epitopes, which can be used as a DC vaccine for CML patients (22,23).

3.2. Dendritic Cells Mobilization Strategies

Due to the low frequency of DC present in the peripheral blood, agents that expand DC numbers *in vivo* could be extremely valuable. Several factors that mobilize peripheral blood DC include GM-CSF, granulocyte-colony stimulating factor (G-CSF), Flt3 ligand (Flt3L) and progenipoietins (Pro-GP). GM-CSF supports DC differentiation and survival, and hence it was one of the first cytokines examined as a DC mobilization agent. Administration of GM-CSF increases the number of CD14+ cells, but does not substantially increase the number of cells with appropriate DC markers or increased antigen presentation properties (27). The combination of GM-CSF and IL-4, however, mobilizes CD14+ cells with phenotypic (e.g., increased HLA-DR and CD11c expression) and functional (increased antigen-presenting activity) properties of DC. (27). A recent report showed that mobilization with G-CSF results in the up-regulation of chemokine receptor CCR7 and down-regulation of CD62L on CD123^{high} blood DC in most healthy donors and myeloma patients, suggesting that G-CSF increases DC counts in part by modulating homing mechanisms (28). A number of other DC mobilization strategies have been discussed in a recent review (29).

3.3. Dendritic Cells Culture Conditions

While DC may be cultured in a variety of media, most recent studies have employed either serum free media or media containing autologous serum. The use of media containing bovine blood components has engendered concern regarding safety issues. For example, DC generated in fetal calf serum have the potential to induce anaphylactic reactions related to a potent IgE response against bovine serum albumin (30). With the ability to reproducibly generate DC *ex vivo* from mononuclear cell precursors, substantial effort has been dedicated to the development of automated cultivation systems for the large-scale preparation of DC products that are safe for clinical applications (31).

3.4. Methods of Loading Dendritic Cells with Tumor Antigens

Many strategies for loading DC with tumor antigens have been described, and there remains no consensus regarding the optimal approach. Recently, approaches that have received the greatest interest include the application of proteins or peptides, apoptotic or necrotic tumor cells, tumor cell lysates, tumor cell nucleic acids, genetically engineered vectors, or cell fusions. This topic has been reviewed recently, and therefore only selected highlights will be briefly discussed below (29,32,33).

Because a large number of tumor antigens and antigenic epitopes have been defined, loading DC with specific tumor antigen proteins or immunodominant peptides is feasible and represents a widely utilized approach. Advantages of loading DC with a specific peptide include the following: (i) because peptide epitopes are precisely defined, specific immune responses may be monitored easily; (ii) the immunodominant epitope(s) can be targeted specifically in an effort to enhance vaccine potency; (iii) most peptides can be produced in large quantities adequate for performance of clinical trials; and (iv) peptide sequences can be modified easily to enhance binding to MHC molecules and/or immunogenicity. The use of whole proteins also offers a number of advantages: (i) proteins may contain multiple immunogenic epitopes; (ii) whole proteins should incorporate both class I and class II restricted epitopes; and (iii) proteins, in contrast to peptides, are not restricted to specific HLA types. Unfortunately, whole protein tumor antigens may be difficult to produce in large quantities.

The use of unfractionated tumor cell products, such as apoptotic tumor cells or lysates of (necrotic) tumor cells, is an attractive method of loading DC for cancer

immunotherapy because such preparations should comprise all expressed tumor antigens. This approach to loading DC with tumor antigens has been utilized in a number of early phase clinical studies (for example, (34-39)). An alternative to the use of unfractionated cell products is the use of exosomes, which are small (60 to 100 nm) vesicles that are spontaneously secreted by a variety of cell types including DC and tumor cells. These vesicles bear antigenic epitopes, class I and class II MHC molecules, costimulatory molecules, and other factors (40). Tumor peptide pulsed DC derived exosomes alone have been shown to stimulate antigen-specific CTL responses in preclinical studies (40,41). Exosomes isolated from malignant effusions or ascites have been shown to bear tumor antigens, and DC pulsed with these exosomes induced the expansion of tumor-specific lymphocytes in peripheral blood mononuclear cells (PBMC) from seven of nine patients (42). Clinical trials investigating exosome-pulsed DC are currently in progress.

Loading DC with tumor-derived nucleic acids (43-47) represents another innovative strategy that may offer a number of advantages: (i) each encoded antigen typically comprises multiple epitopes; (ii) multiple tumor antigens are encoded within tumor-derived nucleic acid preparations; (iii) this strategy is not restricted to one particular HLA type; (iv) both MHC class I and class II epitopes are contained within encoded antigens; and (v) tumor-derived nucleic acid preparations can be prepared in large quantities more readily than most protein antigens. One potential disadvantage of loading tumor-derived nucleic acids onto DC is that, because of the high complexity of genomic material, the quantity of each specific tumor-associated antigen (TAA) that is ultimately available for processing and presentation may be extremely small. Another obstacle is that the amount of tumor tissue, and therefore nucleic acid available may be very small. The quantity of tumor tissue may be particularly limited in patients with minimal tumor burden, or when the ratio of tumor cells to stromal cells in a specimen is very low (for example, following chemotherapy and/or radiation therapy). Recently, however, investigators have made progress toward circumventing this problem by showing that mRNA may be amplified from minute quantities of tumor tissue and loaded onto DC for vaccine preparation (48,49).

A variety of genetically engineered vectors may be used to gene modify DC for tumor antigen presentation, and these have been reviewed previously (50,51). Several viral vectors recently have received particular interest: vaccinia, adenovirus, adeno-associated virus, avipox viruses, retroviruses and lentiviruses. For example, fowlpox, an avipox virus, can infect most human cells but cannot replicate within them, and has attracted interest as a vehicle for delivery of genes into DC (52). DC infected with recombinant vaccinia and fowlpox viruses encoding a TAA have been shown to stimulate anti-melanoma T cell responses *in vitro* (53,54). Recently, a recombinant fowlpox virus expressing three T cell costimulatory molecules (B7.1, ICAM-1 and LFA-3), referred to as rF-TRICOM, has been developed (55). DC infected with rF-TRICOM were shown to hyperexpress all three costimulatory molecules and were found to be more effective stimulators of PSA- and CEA-specific T cell responses than noninfected DC.

An alternative strategy that has been developed for the generation of DC that express tumor antigens is the development of fusions between DC and tumor cells. Preclinical studies have demonstrated that the fusion of DC with tumor cells is feasible and can induce potent tumor-specific immunity (56-60).

3.5. Dendritic Cell Maturation

Interest in DC maturation stems from the rationale that mature DC should function as more potent antigen-presenting cells and stimulators of tumor-specific immunity than immature DC. However, the definition of a "mature" DC remains the subject of ongoing debate and further investigation (61). While the maturation state of DC may be defined phenotypically, based on markers expressed on the cell surface, maturation may also be defined functionally, for example based on the ability to secrete cytokines or induce antigen-specific T cell responses. DC may be matured phenotypically using a variety of stimuli, such as tumor necrosis factor-alpha (TNF-alpha), CD40 ligand (CD40L), Bacillus Calmette-Guerrin (BCG), lipopolysaccharide, calcium ionophores, or other agents. Interestingly, while single agents have the capacity to induce the up-regulation of cell surface maturation markers such as CD83, combinations of signals appear to be necessary to induce full functional maturation as defined by IL-12 production and the ability to induce antigen-specific T cell responses (62-64). Consequently, there has been growing interest in identifying optimal combinations of factors to induce functional DC maturation (61).

The ability to induce phenotypic and functional DC maturation may represent an important index of quality of DC generated for use in immunotherapy trials. In order to develop a method of determining the activity of different lots of IL-4, Colling *et al.* examined several phenotypic and functional parameters of monocyte-derived DC generated *ex vivo* with GM-CSF and IL-4 (65). Dose-response studies showed that, whereas phenotypic markers of maturation typically plateaued at low IL-4 concentrations, allostimulatory capacity and DC IL-12 production in response to maturation signals typically did not plateau until significantly higher concentrations of IL-4 were used for DC generation. Therefore, while the minimum amount of IL-4 needed for DC generation may be determined by phenotypic parameters, consideration of functional maturation may be important in optimizing IL-4 concentration.

Further highlighting the complexity of the DC maturation, not only are there a multiple types of signals that may trigger phenotypic and/or functional maturation (63,66-72), but the sequence and time of signals may be critical (61). Vegh recently reported that the *ex vivo* generated DC could be functionally matured by loading them with tumor lysate for four hours followed by

treatment with TNF-alpha plus IFN-gamma for 24 hours (73). After 48 hours of treatment, however, IL-12 production by DC had declined.

3.6. The Role of Helper Antigens

While the role of CTL in the generation of effective antitumor immunity has received great emphasis, the importance of T cell help is increasingly being appreciated. Using DC loaded with lysates of a pancreatic carcinoma cells line, Schnurr *et al.* showed that tumor-specific cytolytic and Th1-type cellular responses could be generated and were more pronounced when keyhole limpet hemocyanin (KLH) was added to the lysate (74). This work suggests that helper T cell epitopes contained within KLH may enhance the ability of DC to induce CD8+ CTL responses.

3.7. Cryopreservation of Dendritic Cells

While the cryopreservation of biospecimens is has been widely practiced in clinical medicine and biomedical research, the impact of this process on cell viability and particularly function sometimes may be underestimated. The routine freezing of DC prior to use in cancer vaccine trials should be viewed with caution for two reasons: (i) there are no standardized criteria for rigorously measuring the quality of DC to be used in vaccine preparations; and (ii) the full impact of cryopreservation and subsequent thawing on DC function has not been precisely established. Lewalle *et al.* found that cryopreservation and thawing of DC generated from normal volunteers did not affect their ability to take up and present antigen *in vitro*, stimulate allogeneic CD8+ T cell responses, or elaborate IL-12 in response to maturation factors (75). More recently, Westermann and colleagues also investigated whether cryopreservation and thawing of DC alters their phenotypic or functional properties (76). They analyzed DC from healthy volunteers and from patients with CML for viability, morphology, immunophenotype, capacity for allostimulation in a mixed lymphocyte reaction (MLR), and mobility (by time-lapse cinemicroscopy). They found no significant effect of cryopreservation and thawing on the phenotype or function of DC, whether generated from healthy volunteers or CML patients. However, John and colleagues found that previously cryopreserved DC had decreased endocytic activity, efficiency of adenoviral infection in gene transfer studies, and IL-12 production following maturation (77). Therefore, it remains unclear whether cryopreservation significantly impacts processes that are important for DC function, and a formal comparison of between fresh DC and previously cryopreserved DC in the context of immunotherapy trials may be necessary to resolve this issue.

4. ADMINISTRATION OF DENDRITIC CELL VACCINES

Many variables may influence the potency of DC-based vaccines. Among the factors that have received particular interest are the route of administration, frequency of immunizations, administration of adjuvant immune-enhancing agents, and prime-boost vaccination strategies. These topics are briefly discussed below.

4.1. Route of Dendritic Cell Administration

Investigators have administered DC intravenously (IV), intradermally, subcutaneously, intralymphatically, intranodally and intratumorally in an effort to determine the best route. Which route is optimal for administration of DC vaccines remains the focus of ongoing investigation, and a complete review of the published literature on this topic is beyond the scope of this article.

One reason that the route of administration has received significant attention is that the generation of potent antigen-specific immune responses appears to depend on proper trafficking of antigen-loaded DC to the site(s) of antigen presentation. In an effort to study the trafficking of DC following administration, Morse and colleagues administered indium-labeled monocyte-derived DC by intravenous, subcutaneous, or intradermal injection (78). DC injected IV accumulated in the lungs and subsequently redistributed to the liver, spleen and bone marrow, but were not detected in lymph nodes or tumors. A small percentage of DC injected intradermally localized to regional lymphatics in some cases, whereas accumulation of tracer was noted in the lymph nodes after subcutaneous injection.

Fong and colleagues treated 21 metastatic prostate cancer patients with autologous DC activated and cocultured *ex vivo* with recombinant mouse prostatic acid phosphatase (79). Following activation, DC were noted to have up-regulation of maturation markers (e.g., CD80 and CD83), while maintaining expression of adhesion molecules (e.g., CD44 and LFA-1). In contrast, they found that CD62 ligand and CCR5 expression was down-regulated. They hypothesized that if the DC were administered IV, the absence of CD62 ligand would impair the ability to prime T cells by prohibiting access to lymphoid tissue via the high endothelial venules. All patients mounted antigen-specific immune responses, but intradermal and intralymphatic admininstration induced IFN-gamma responses, while the IV route preferentially induced humoral responses. The results suggest that, while DC may have the capacity to induce antigen-specific T cell responses regardless of the route of administration, the nature of the immune response may vary substantially depending on which route is used.

4.2. Immunization Schedule

The optimal dose (number of DC) and frequency of immunization with DC-based vaccines also remain unclear despite a number of investigations that have addressed immunization schedule. In a murine model, one group found that multiple immunizations with DC transduced with the MART-1 gene resulted in a shift toward a Th2 cytokine profile and poorer protection against tumor challenge than did a single immunization (80). Interestingly, the adverse effect of multiple immunizations was not present in Fas receptor knockout mice, suggesting a possible role for Fas receptor-mediated clearance of antigen-specific interferon-gamma (IFN-gamma) producing T cells in response to multiple immunizations. The dose

and frequency of DC vaccine administration are being examined in ongoing clinical trials.

4.3. Use of Vaccine Adjuvants

A variety of adjuvant agents have been administered in conjunction with DC-based cancer vaccines in an effort to enhance the robustness of tumor-specific immune responses. Adjuvants may be administered locally with a vaccine preparation or systemically. It appears that a number of vaccine adjuvants act primarily through the functional maturation of DC such as BCG (81) and/or the provision of helper T cell (particularly Th1) signals such as CD40L (82,83) (see *Section 3.5*, above). The identification and characterization of a wide array of other immune-enhancing agents with various mechanisms of action represents an area of intense interest.

Examples of vaccine adjuvants that have received significant attention include bacterial products (e.g., BCG, lipopolysaccharide), lipid-based preparations (e.g., incomplete Freund's adjuvant, montanide ISA-51), cytokines (e.g., IL-2, GM-CSF, IL-12), immunostimulatory nucleic acids (e.g., CpG-rich oligonucleotides), and heat-shock proteins. A number of these adjuvants have been incorporated into DC vaccine trials.

Recently there has been particularly strong interest in using cytokine adjuvants in conjunction with DC vaccines (84). Interest in the use of IL-2 as a vaccine adjuvant stems in part from its well described activity against metastatic melanoma and renal cell carcinoma, but high-dose systemic IL-2 therapy may be associated with severe toxicity. Rubinstein and colleagues have investigated IL-15 as a vaccine adjuvant, reasoning that it has similar activities to IL-2, but has a more favorable toxicity profile (85). Using a murine model, this group showed that IL-15 is capable of augmenting primary antigen-specific CTL responses to DC vaccination. Another innovative strategy is to provide a vaccine adjuvant through genetic modification of DC. Zitvogel and colleagues showed in a murine model that retroviral transduction or plasmid transfection procedures can be used to induce high-level expression of IL-12 in DC (86). These engineered cells were able to enhance antitumor CTL responses.

4.4. Prime-Boost Strategy

One problem that may be associated with the use of more complex DC preparations is that immune responses against non-TAA epitopes could predominate over those against TAA epitopes. For example, following multiple immunizations with a viral vector modified DC, cellular immune responses against foreign viral antigens could potentially compromise competing responses against encoded tumor antigens. Perhaps more concerning, neutralizing antiviral antibodies may abrogate the ability of subsequent immunizations to boost anti-TAA immune responses. A strategy that could help circumvent this problem is to prime with one DC vaccine preparation and boost with a heterologous preparation (prime-boost approach) (87).

Tuttenberg *et al.* found that DC infected with an adenoviral construct encoding the melanoma gp100 antigen could induce potent antigen-specific T cell responses against multiple gp100 epitopes *in vitro* associated with high levels of IL-2 and IFN-gamma release (88). Interestingly, they found that repeated restimulation resulted in a diminution of the gp100-specific response along with a parallel increase in the anti-adenoviral T-cell response. They proposed that a prime-boost immunization strategy incorporating a combination of peptide pulsed DC and adenoviral vector modified DC may result in long-lasting antitumor T cell responses.

5. CLINICAL TRIALS EVALUATING DC-BASED CANCER VACCINES

A large number of early phase clinical trials have been undertaken to evaluate the application of DC vaccines to the treatment of a variety of advanced malignancies. Several clinical trials will be discussed briefly below in order to highlight some of the recent advancements in the application of DC-based antitumor immunotherapy.

5.1. Malignant Melanoma

Malignant melanoma continues to be a fertile area for the conduct of immunotherapy trials, including DC-based vaccine trials. Lau *et al.* administered IV immunizations with monocyte-derived DC pulsed with two HLA-A*0201-restricted melanoma peptides (tyrosinase(368-376:370D) and gp100(209-217:210M)) to sixteen patients with metastatic melanoma (89). The vaccine was generally well-tolerated. One patient had complete remission of lung and pleural disease, two patients had stable disease, and two patients had mixed responses. Five patients had an immune response to gp100 or tyrosinase by IFN-gamma release, and four of the five patients had tumor regression or disease stabilization, indicating concordance of immunologic and clinical responses.

Banchereau and colleagues immunized 18 metastatic melanoma patients using subcutaneous injections of CD34+ progenitor-derived autologous dendritic cells pulsed with four HLA-A*0201-restricted melanoma peptides (MART-1, tyrosinase, MAGE-3, and gp100), as well as influenza matrix peptide and KLH as control antigens (90). Two patients developed progressive vitiligo, but the vaccine was generally well tolerated. Sixteen of 18 patients developed an immune response to the control antigens and an enhanced immune response to one or more of the melanoma peptides. Interestingly, the investigators found a significant association between clinical progression and an immunologic response to two or fewer melanoma peptides.

Another research team generated autologous monocyte-derived DC and fused these DC with gamma-irradiated primary autologous tumor cells by incubation in polyethylene glycol (91). They vaccinated seventeen patients subcutaneously at monthly intervals without major toxicity. One patient had a partial response, one patient progressed but had regression of some deposits, and one patient exhibited disease stabilization for six months.

Smithers and colleagues immunized 19 metastatic melanoma patients intradermally with DC loaded with acid-eluted autologous melanoma peptide and particulate hepatitis B surface antigen (HBsAg) (92). Toxicity was limited to the development of flu-like symptoms, vitiligo, or autoantibodies. Four of the nine patients who exhibited cellular responses to HBsAg (HBsAg responders) had objective clinical responses or disease stabilization, while none of the ten patients who failed to exhibit such an immune response (HBsAg nonresponders) had clinical responses. Five of the nine HBsAg responders exhibited a melanoma peptide specific IFN-gamma response, while only one of the nonresponders did so. Therefore, it appears that the use of control particulate antigens to gauge immune responsiveness may play a valuable role in the design of DC vaccine trials.

5.2. Hematologic Malignancies

Some hematologic malignancies, such as multiple myeloma and B cell lymphoma, express monoclonal immunoglobulins with specific antigenic determinants, or idiotypes (Id). These idiotypes, which are unique and uniform in each individual patient, may be treated as TAA that can be targeted by specific immunotherapy. Hsu and colleagues treated four follicular B-cell lymphoma patients with autologous DC pulsed *ex vivo* with tumor-specific idiotype protein (11). They observed clinical responses in three patients and tumor-specific cellular immune responses in all four patients.

Several clinical trials have examined the application of DC-based vaccines to multiple myeloma and have been reviewed elsewhere (93). In one study, Titzer and colleagues treated eleven patients with advanced myeloma with CD34 stem cell-derived DC pulsed with Id peptides (94). Increased anti-idiotype antibody serum titers were noted in three out of ten patients, and increased Id-specific T cell activity was observed in four out of ten patients by ELISpot immunologic analysis. In addition one patient exhibited a decrease in bone marrow plasma cell infiltration.

Liso and colleagues treated 25 myeloma patients with high-dose chemotherapy (HDC) and peripheral blood progenitor cell transplantation (PBPCT) and DC-based Id protein vaccination (95). Following HDC-PBPCT, patients received intravenous infusions of DC loaded either with Id protein or with Id coupled with KLH, followed by subcutaneous boosts of Id-KLH conjugates. Twenty-four of 26 patients developed a KLH-specific cellular proliferative response, while only 4 patients showed an Id-specific proliferative response. Reichardt and colleagues treated twelve multiple myeloma patients with high-dose therapy and peripheral blood stem cell transplantation (PBSCT) followed by Id-pulsed DC and Id/KLH immunizations (10). Eleven of twelve patients developed KLH-specific cellular proliferative responses, two of twelve patients had Id-specific response, and one of three patients evaluated had a transient Id-specific CTL response. The two patients who developed an Id-specific cellular response were among the nine patients who had a complete remission, and these two patients remained in complete remission following vaccination. This study demonstrated that DC-based Id vaccination is feasible after HDC-PBSCT and that these patients can mount KLH- and Id-specific T cell responses.

5.3. Genitourinary Tract Malignancies

Small and colleagues performed a sequential phase I/II clinical trial in hormone-refractory prostate cancer patients immunized intravenously with Provenge (Dendreon Corp, Seattle, WA), a preparation of autologous dendritic cells loaded *ex vivo* with a recombinant prostatic acid phosphatase-GM-CSF fusion protein (12). The most common adverse event was fever, but the immunizations were generally well tolerated. Immune responses against the fusion protein were observed in all patients, and immune responses against prostatic acid phosphatase were observed in 38%. Six patients were noted to have substantial declines in PSA. The investigators noted a correlation of time to disease progression with the generation of an immune response against PAP and with the dose of dendritic cells administered.

Su *et al.* conducted a phase I trial of monocyte-derived DC transfected with total tumor RNA in ten evaluable patients with metastatic renal cell carcinoma (96). They observed an acceptable toxicity profile, and six of seven evaluable subjects demonstrated expansion of tumor-specific T cells following immunization. Interestingly, the T cell responses detected in these patients were directed against renal TAA such as telomerase and oncofetal antigen, but not against normal renal self-antigens.

Cervical cancer seems particularly suitable to treatment with cancer vaccines because of the well-documented etiologic role of human papillomavirus (HPV) infection (97). Demonstration that immunization of young women with a viral particle-like vaccine against HPV type 16 (HPV16) protects against the development of cervical intraepithelial neoplasia supports this concept (98). The effectiveness of this approach may be related to the ability of HPV16 viral-like particles to induce maturation and activation of DC (99). Santin *et al.* have shown that DC pulsed with HPV16 and HPV18 E7 oncoprotein stimulates tumor-specific cytotoxicity induce antigen-specific CTL responses in PBMC from healthy patients (100) and in populations of tumor infiltrating lymphocytes (TIL) from cervical cancer patients (101). Clinical trials evaluating DC-based vaccines for the treatment of established cervical cancer are in progress.

Hernando *et al.* recently reported a phase I clinical trial of intradermal immunizations with DC pulsed with KLH and autologous tumor lysates in two patients with uterine sarcoma and six patients with ovarian cancer (102). Three patients had disease stabilization, and five had tumor progression within the first 14 weeks. While all except one patient exhibited immune responses to KLH, responses to tumor lysate were detected in only one patient by DTH reactivity, two patients by proliferation assay, and one patient by interferon-gamma release. Nonetheless, the study demonstrated the safety and feasibility of tumor

lysate loaded DC in patients with advanced gynecologic malignancies.

5.4. Gastrointestinal Malignancies

Kono and colleagues performed a phase I trial to examine DC vaccine therapy in metastatic gastric cancer (103). Nine HLA A2+ patients with HER-2/neu overexpressing gastric cancers were immunized intradermally with DC pulsed with HER-2(p369) peptide. The immunizations were well tolerated, and one patient had a partial clinical response while another had disease stabilization for three months. HER-2/neu-specific T cell responses developed in six of the nine patients by IFN-gamma release, and CTL activity was demonstrated in two patients. Therefore, DC vaccine therapy is safe and feasible in advanced gastric cancer patients and can induce HER-2/neu-specific Th1 type cellular responses.

Another group explored the used of DC immunotherapy in ten patients with unresectable primary liver cancer (hepatocellular carcinoma or cholangiocarcinoma) (39). Monocyte-derived DC were generated *ex vivo*, were pulsed with autologous tumor lysate, TNF-alpha and KLH, and were incubated for nine days. Non-adherent cells were harvested and were injected into inguinal lymph nodes. Patients tolerated the regimen well, but there was only one mixed clinical response and two responses manifest as a decrease in tumor markers following immunization. Seven of ten patients did show DTH reactivity to KLH. The study demonstrated the safety and feasibility of immunotherapy in this group of patients, but acknowledged that further modification of the vaccine to enhance bioactivity would appear to be indicated.

5.5. Other Malignancies

Yu and colleagues performed a phase I trial of DC immunotherapy in seven patients with glioblastoma multiforme and two patients with anaplastic astrocytoma (104). Patients received intradermal immunizations with autologous peripheral blood dendritic cells pulsed with peptides eluted from the surface of autologous glioma cells. The investigators observed intratumoral cytotoxic and memory T-cell infiltration in two of four patients who underwent reoperation following DC immunization.

Brossart *et al.* vaccinated advanced breast and ovarian cancer patients with autologous DC pulsed with HER-2/neu- or MUC1-derived peptides (105). Peptide-specific CD8+ T cell responses could be detected in the peripheral blood in five out of ten patients based on ^{51}Cr-release assays and intracellular IFN-gamma staining. Responses against HER-2/neu-derived E75 and the MUC1-derived M1.2 peptide were particularly robust and durable (lasting longer than 6 months), prompting the authors to postulate that these peptides represent immunodominant epitopes. Interestingly, two patients appeared to develop immune responses against tumor antigens other than those contained within the vaccine, suggesting that antigen spreading occurs in some patients who mount significant T cell responses to cancer vaccines.

Stift *et al.* immunized 20 patients with various stage IV malignancies (pancreatic cancer, hepatocellular carcinoma, cholangiocarcinoma, and medullary thyroid carcinoma) using autologous tumor lysate loaded DC (106). To prepare the vaccine, they isolated CD14+ monocytes by magnetic bead separation, generated DC *ex vivo* with GM-CSF and IL-4, pulsed DC with autologous tumor lysate, and matured them with TNF-alpha. DC were administered intranodally by ultrasound guidance, and patients received adjuvant systemic IL-2 following each immunization. While the vaccination procedure was well tolerated, there were no complete or partial responses. However, a number of patients did show objective evidence of a clinical response manifest as either a decrement in tumor markers or a mixed response of measurable tumor deposits. In addition, 18 patients had positive DTH responses and three patients had antigen-specific cellular responses by IFN-gamma release. Therefore, this strategy of intranodal DC immunization in conjunction with systemic IL-2 treatment was feasible and well tolerated and appears to exhibit some biological activity in patients with advanced malignancies.

6. PERSPECTIVE

Sophisticated methods of preparing dendritic cell based cancer vaccines have been developed and characterized in preclinical studies. The safety and feasibility of these approaches have been documented in a number of studies. Several ongoing challenges remain before truly efficacious dendritic cell vaccines will be available for the treatment of cancer. The first is to understand the key immunoregulatory processes at the molecular level so that they can be modulated in a predictable manner. The second is to identify, optimize and determine the appropriate combination of molecular signals that results in the induction of potent and clinically significant antitumor immunity. The third is to develop standard cellular processing and immune monitoring methodologies that will make pivotal multi-center cancer vaccine trials feasible. Finally, the best vaccine candidates and most appropriate subset(s) of patients in whom to test each vaccine must be identified for testing in large-scale trials.

7. ACKNOWLEDGMENTS

The authors would like to thank all the members of the Program in Molecular Therapeutics in the Duke Comprehensive Cancer Center. This work was supported by: NIH 1R01CA095846-01 (H.K.L.), NIH M01RR00030 (M.A.M.), and NIH 1R01CA95447-01A1 (T.M.C.). M.A.M. is a recipient of an American Society of Clinical Oncology Career Development Award.

8. REFERENCES

1. Carbone JE, DP Ohm: Immune dysfunction in cancer patients. *Oncol* 16, 11-18 (2002)
2. Chen HL, D Gabrilovich, A Virmani, I Ratnani, KR Girgis, S Nadaf-Rahrov, M Fernandez-Vina, DP Carbone: Structural and functional analysis of beta2 microglobulin

abnormalities in human lung and breast cancer. *Int J Cancer* 67, 756-763 (1996)
3. Morse MA, HK Lyerly: Clinical applications of dendritic cell vaccines. *Curr Opin Mol Ther* 2, 20-28 (2000)
4. Wu L, S Vandenabeele, K Georgopoulos: Derivation of dendritic cells from myeloid and lymphoid precursors. *Int Rev Immunol* 20, 117-135 (2001)
5. Galibert L, CR Maliszewski, S Vandenabeele: Plasmacytoid monocytes/T cells: a dendritic cell lineage? *Sem Immunol* 13, 283-289 (2001)
6. Liu YJ, H Kanzler, V Soumelis, M Gilliet: Dendritic cell lineage, plasticity and cross-regulation. *Nature Immunol* 2, 585-589 (2001)
7. Moser M, KM Murphy: Dendritic cell regulation of TH1-TH2 development. *Nature Immunol* 1, 199-205 (2000).
8. Lanzavecchia A, F Sallusto: The instructive role of dendritic cells on T cell responses: lineages, plasticity and kinetics. *Curr Opin Immunol* 13, 291-298 (2001)
9. Fearnley DB, LF Whyte, SA Carnoutsos, AH Cook, DN Hart: Monitoring human blood dendritic cell numbers in normal individuals and in stem cell transplantation. *Blood* 93, 728-736 (1999)
10. Reichardt VL, CY Okada, A Liso, CJ Benike, KE Stockerl-Goldstein, EG Engleman, KG Blume, R Levy: Idiotype vaccination using dendritic cells after autologous peripheral blood stem cell transplantation for multiple myeloma--a feasibility study. *Blood* 93, 2411-2419 (1999)
11. Hsu FJ, C Benike, F Fagnoni, TM Liles, D Czerwinski, B Taidi, EG Engleman, R Levy: Vaccination of patients with B-cell lymphoma using autologous antigen-pulsed dendritic cells. *Nature Med* 2, 52-58 (1996)
12. Small EJ, P Fratesi, DM Reese, G Strang, R Laus, MV Peshwa, FH Valone: Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. *J Clin Oncol* 18, 3894-3903 (2000)
13. Gabrilovich DI, J Corak, IF Ciernik, D Kavanaugh, DP Carbone: Decreased antigen presentation by dendritic cells in patients with breast cancer. *Clin Cancer Res* 3, 483-490 (1997)
14. Ratta M, F Fagnoni, A Curti, R Vescovini, P Sansoni, B Oliviero, M Fogli, E Ferri, GR Della Cuna, S Tura, M Baccarani, RM Lemoli: Dendritic cells are functionally defective in multiple myeloma: the role of interleukin-6. *Blood* 100, 230-237 (2002)
15. Romani N, S Gruner, D Brang, E Kampgen, A Lenz, B Trockenbacher, G Konwalinka, PO Fritsch, RM Steinman, G Schuler: Proliferating dendritic cell progenitors in human Blood *J Exp Med* 180, 83-93 (1994)
16. Cao H, V Verge, C Baron, C Martinache, A Leon, S Scholl, NC Gorin, J Salamero, S Assari, J Bernard, M Lopez: *In vitro* generation of dendritic cells from human blood monocytes in experimental conditions compatible for *in vivo* cell therapy. *J Hematother Stem Cell Res* 9, 183-194 (2000)
17. Morse MA, HK Lyerly, Y Li: The role of IL-13 in the generation of dendritic cells *in vitro*. *J Immunother* 22, 506-513 (1999)
18. Alters SE, JR Gadea, B Holm, J Lebkowski, R Philip: IL-13 can substitute for IL-4 in the generation of dendritic cells for the induction of cytotoxic T lymphocytes and gene therapy. *J Immunother* 22, 229-236 (1999)
19. Mortarini R, A Anichini, M Di Nicola, S Siena, M Bregni, F Belli, A Molla, AM Gianni, G Parmiani: Autologous dendritic cells derived from CD34+ progenitors and from monocytes are not functionally equivalent antigen-presenting cells in the induction of melan-A/Mart-1(27-35)-specific CTLs from peripheral blood lymphocytes of melanoma patients with low frequency of CTL precursors. *Cancer Res* 57, 5534-5541 (1997)
20. Ferlazzo G, A Wesa, WZ Wei, A Galy: Dendritic cells generated either from CD34+ progenitor cells or from monocytes differ in their ability to activate antigen-specific CD8+ T cells. *J Immunol* 163, 3597-3604 (1999)
21. Ferlazzo G, J Klein, X Paliard, WZ Wei, A Galy: Dendritic cells generated from CD34+ progenitor cells with flt3 ligand, c-kit ligand, GM-CSF, IL-4, and TNF-alpha are functional antigen-presenting cells resembling mature monocyte-derived dendritic cells. *J Immunother* 23, 48-58 (2000)
22. Smit WM, M Rijnbeek, CA van Bergen, RA de Paus, HA Vervenne, M van de Keur, R Willemze, JH Falkenburg: Generation of dendritic cells expressing bcr-abl from CD34-positive chronic myeloid leukemia precursor cells. *Hum Immunol* 53, 216-223 (1997)
23. Westermann J, J Kopp, I Korner, G Richter, Z Qin, T Blankenstein, B Dorken, A Pezzutto: Bcr/abl+ autologous dendritic cells for vaccination in chronic myeloid leukemia. *Bone Marrow Transplant* 25, S46-49 (2000)
24. Cignetti A, E Bryant, B Allione, A Vitale, R Foa, MA Cheever: CD34(+) acute myeloid and lymphoid leukemic blasts can be induced to differentiate into dendritic cells. *Blood* 94, 2048-2055 (1999)
25. Narita M, M Takahashi, A Liu, K Nikkuni, T Furukawa, K Toba, S Koyama, K Takai, M Sanada, Y Aizawa: Leukemia blast-induced T-cell anergy demonstrated by leukemia-derived dendritic cells in acute myelogenous leukemia. *Exp Hematol* 29, 709-719 (2001)
26. Westers TM, AG Stam, RJ Scheper, JC Regelink, AW Nieuwint, GJ Schuurhuis, AA van de Loosdrecht, GJ Ossenkoppele: Rapid generation of antigen-presenting cells from leukaemic blasts in acute myeloid leukaemia. *Cancer Immunol Immunother* 52, 17-27 (2003)
27. Roth MD, BJ Gitlitz, SM Kiertscher, AN Park, M Mendenhall, N Moldawer, RA Figlin: Granulocyte macrophage colony-stimulating factor and interleukin 4 enhance the number and antigen-presenting activity of circulating CD14+ and CD83+ cells in cancer patients. *Cancer Res* 60, 1934-1941 (2000)
28. Vuckovic S, M Kim, D Khalil, CJ Turtle, GV Crosbie, N Williams, L Brown, K Williams, C Kelly, P Stravos, R Rodwell, GR Hill, S Wright, K Taylor, D Gill, P Marlton, K Bradstock, DN Hart: Granulocyte-colony stimulating factor increases CD123hi blood dendritic cells with altered CD62L and CCR7 expression. *Blood* 101, 2314-2317 (2003).
29. Mosca PJ, TM Clay, HK Lyerly, MA Morse: Current status of dendritic cell immunotherapy of malignancies. *Intern Rev Immunol* 22, 255-281 (2003)
30. Mackensen A, R Drager, M Schlesier, R Mertelsmann, A Lindemann: Presence of IgE antibodies to bovine serum albumin in a patient developing anaphylaxis after

vaccination with human peptide-pulsed dendritic cells. *Cancer Immunol Immunother* 49, 152-156 (2000)
31. Pullarkat V, R Lau, SM Lee, JG Bender, JS Weber: Large-scale monocyte enrichment coupled with a closed culture system for the generation of human dendritic cells. *J Immunol Methods* 267, 173-183 (2002)
32. Strome SE, S Voss, R Wilcox, TL Wakefield, K Tamada, D Flies, A Chapoval, J Lu, JL Kasperbauer, D Padley, R Vile, D Gastineau, P Wettstein, L Chen: Strategies for antigen loading of dendritic cells to enhance the antitumor immune response. *Cancer Res* 62, 1884-1889 (2002)
33. Zhou Y, ML Bosch, ML Salgaller: Current methods for loading dendritic cells with tumor antigen for the induction of antitumor immunity. *J of Immunotherapy* 25, 289-303 (2002)
34. Nestle FO, S Alijagic, M Gilliet, Y Sun, S Grabbe, R Dummer, G Burg, D Schadendorf: Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nature Med* 4, 328-332 (1998)
35. Geiger JD, RJ Hutchinson, LF Hohenkirk, EA McKenna, GA Yanik, JE Levine, AE Chang, TM Braun, JJ Mule: Vaccination of pediatric solid tumor patients with tumor lysate-pulsed dendritic cells can expand specific T cells and mediate tumor regression. *Cancer Res* 61, 8513-8519 (2001)
36. Azuma T, S Horie, K Tomita, T Takahashi, Y Tanaka, K Kashiwase, M Nieda, T Takeuchi, N Ohta, Y Shibata, H Hirai, T Kitamura: Dendritic cell immunotherapy for patients with metastatic renal cell carcinoma: University of Tokyo experience. *Int J of Urology* 9, 340-346 (2002)
37. Chang AE, BG Redman, JR Whitfield, BJ Nickoloff, TM Braun, PP Lee, JD Geiger, JJ Mule: A phase I trial of tumor lysate-pulsed dendritic cells in the treatment of advanced cancer. *Clin Cancer Res* 8, 1021-1032 (2002)
38. Holtl L, C Zelle-Rieser, H Gander, C Papesh, R Ramoner, G Bartsch, H Rogatsch, AL Barsoum, JH Coggin, Jr., M Thurnher: Immunotherapy of metastatic renal cell carcinoma with tumor lysate-pulsed autologous dendritic cells. *Clin Cancer Res* 8, 3369-3376 (2002)
39. Iwashita Y, K Tahara, S Goto, A Sasaki, S Kai, M Seike, CL Chen, K Kawano, S Kitano: A phase I study of autologous dendritic cell-based immunotherapy for patients with unresectable primary liver cancer. *Cancer Immunol Immunother* 52, 155-161 (2003)
40. Zitvogel L, A Regnault, A Lozier, J Wolfers, C Flament, D Tenza, Ricciardi-Castagnoli P, Raposo G, Amigorena S: Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nature Med* 4:594-600 (1998)
41. Amigorena S: Cancer immunotherapy using dendritic cell-derived exosomes. *Medicina* 60, 51-54 (2000)
42. Andre F, NE Schartz, M Movassagh, C Flament, P Pautier, P Morice, C Pomel, C Lhomme, B Escudier, T Le Chevalier, T Tursz, S Amigorena, G Raposo, E Angevin, L Zitvogel: Malignant effusions and immunogenic tumour-derived exosomes. *Lancet* 360, 295-305 (2002)
43. Boczkowski D, SK Nair, D Snyder, E Gilboa: Dendritic cells pulsed with RNA are potent antigen-presenting cells *in vitro* and *in vivo*. *J Exp Med* 184, 465-472 (1996)
44. Mitchell DA, SK Nair: RNA transfected dendritic cells as cancer vaccines. *Curr Opin Mol Ther* 2, 176-181 (2000)
45. Van Tendeloo VF, P Ponsaerts, F Lardon, G Nijs, M Lenjou, Van C Broeckhoven, DR Van Bockstaele, ZN Berneman: Highly efficient gene delivery by mRNA electroporation in human hematopoietic cells: superiority to lipofection and passive pulsing of mRNA and to electroporation of plasmid cDNA for tumor antigen loading of dendritic cells. *Blood*, 98, 49-56 (2001)
46. Strobel I, S Berchtold, A Gotze, U Schulze, G Schuler, A Steinkasserer: Human dendritic cells transfected with either RNA or DNA encoding influenza matrix protein M1 differ in their ability to stimulate cytotoxic T lymphocytes. *Gene Ther* 7, 2028-2035 (2000)
47. Nair SK, D Boczkowski, M Morse, RI Cumming, HK Lyerly, E Gilboa: Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes *in vitro* using human dendritic cells transfected with RNA. *Nature Biotech* 16, 364-369 (1998)
48. Heiser A, MA Maurice, DR Yancey, NZ Wu, P Dahm, SK Pruitt, D Boczkowski, SK Nair, MS Ballo, E Gilboa, J Vieweg: Induction of polyclonal prostate cancer-specific CTL using dendritic cells transfected with amplified tumor RNA. *J Immunol* 166, 2953-2960 (2001)
49. Boczkowski D, SK Nair, JH Nam, HK Lyerly, E Gilboa: Induction of tumor immunity and cytotoxic T lymphocyte responses using dendritic cells transfected with messenger RNA amplified from tumor cells. *Cancer Res* 60, 1028-1034 (2000)
50. Cusack Jr JC, KK Tanabe: Cancer gene therapy. *Surg Oncol Clinics North Am* 7, 421-469 (1998)
51. Bubenik J: Genetically engineered dendritic cell-based cancer vaccines. *Int J Oncol* 18, 475-478 (2001)
52. Brown M, Y Zhang, S Dermine, EA de Wynter, C Hart, H Kitchener, PL Stern, MA Skinner, SN Stacey: Dendritic cells infected with recombinant fowlpox virus vectors are potent and long-acting stimulators of transgene-specific class I restricted T lymphocyte activity. *Gene Ther* 7, 1680-1689 (2000)
53. Yang S, D Kittlesen, CL Slingluff, Jr., CE Vervaert, HF Seigler, TL Darrow: Dendritic cells infected with a vaccinia vector carrying the human gp100 gene simultaneously present multiple specificities and elicit high-affinity T cells reactive to multiple epitopes and restricted by HLA-A2 and -A3. *J Immunol* 164, 4204-4211 (2000)
54. Kim CJ, J Cormier, M Roden, L Gritz, GP Mazzara, P Fetsch, Y Hijazi, KH Lee, SA Rosenberg, FM Marincola: Use of recombinant poxviruses to stimulate anti-melanoma T cell reactivity. *Ann Surg Oncol* 5, 64-76 (1998)
55. Zhu M, H Terasawa, J Gulley, D Panicali, P Arlen, J Schlom, KY Tsang: Enhanced activation of human T cells via avipox vector-mediated hyperexpression of a triad of costimulatory molecules in human dendritic cells. *Cancer Res* 61, 3725-3734 (2001)
56. Gong J, D Chen, M Kashiwaba, D Kufe: Induction of antitumor activity by immunization with fusions of dendritic and carcinoma cells. *Nature Med* 3, 558-561 (1997)
57. Gong J, N Nikrui, D Chen, S Koido, Z Wu, Y Tanaka, S Cannistra, D Avigan, D Kufe: Fusions of human ovarian carcinoma cells with autologous or allogeneic dendritic cells induce antitumor immunity. *J Immunol* 165, 1705-1711 (2000)

58. Li J, LM Holmes, KJ Franek, KE Burgin, TE Wagner, Y Wei: Purified hybrid cells from dendritic cell and tumor cell fusions are superior activators of antitumor immunity. *Cancer Immunol Immunother* 50, 456-462 (2001).
59. Jantscheff P, G Spagnoli, P Zajac, CF Rochlitz: Cell fusion: an approach to generating constitutively proliferating human tumor antigen-presenting cells. *Cancer Immunol Immunother* 51, 367-375 (2002)
60. Xia J, Tanaka Y, Koido S, Liu C, Mukherjee P, Gendler SJ, Gong J: Prevention of spontaneous breast carcinoma by prophylactic vaccination with dendritic/tumor fusion cells. *J Immunol* 2003, 170:1980-1986.
61. Morse MA, PJ Mosca, TM Clay, HK Lyerly: Dendritic cell maturation in active immunotherapy strategies. *Expert Opin Biol Ther* 2, 35-43 (2002).
62. Snijders A, P Kalinski, CM Hilkens, ML Kapsenberg: High-level IL-12 production by human dendritic cells requires two signals. *Int Immunol* 10, 1593-1598 (1998)
63. Mosca PJ, AC Hobeika, TM Clay, SK Nair, EK Thomas, MA Morse, HK Lyerly: A subset of human monocyte-derived dendritic cells expresses high levels of interleukin-12 in response to combined CD40 ligand and interferon-gamma treatment. *Blood* 96, 3499-3504 (2000)
64. Mosca PJ, AC Hobeika, TM Clay, EK Thomas, MA Morse, HK Lyerly: Multiple signals are required for maturation human dendritic cells mobilized *in vivo* with Flt3-Ligand. *J Leuk Biol* 72, 546-53 (2002)
65. Colling KL, A Hobeika, P Mosca, TM Clay, HK Lyerly, MA Morse: Measuring the activity of IL-4 used to generate monocyte-derived dendritic cells for Clin trials. *Preclinica* March/April, 51-57 (2003)
66. Cella M, F Sallusto, A Lanzavecchia: Origin, maturation and antigen presenting function of dendritic cells. *Curr Opin Immunol* 9, 10-16 (1997)
67. Canque B, S Camus, M Yagello, JC Gluckman: IL-4 and CD40 ligation affect differently the differentiation, maturation, and function of human CD34+ cell-derived CD1a+CD14- and CD1a-CD14+ dendritic cell precursors *in vitro*. *J Leuk Biol* 64, 235-244 (1998)
68. Kalinski P, JH Schuitemaker, CM Hilkens, ML Kapsenberg: Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a+CD83+ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. *J Immunol* 161, 2804-2809 (1998)
69. Askew D, RS Chu, AM Krieg, CV Harding: CpG DNA induces maturation of dendritic cells with distinct effects on nascent and recycling MHC-II antigen-processing mechanisms. *J Immunol* 165, 6889-6895 (2000)
70. Kato K, Y Takaue, H Wakasugi: T-cell-conditioned medium efficiently induces the maturation and function of human dendritic cells. *J Leuk Biol* 70, 941-949 (2001)
71. Kuppner MC, R Gastpar, S Gelwer, E Nossner, O Ochmann, A Scharner, RD Issels: The role of heat shock protein (hsp70) in dendritic cell maturation: hsp70 induces the maturation of immature dendritic cells but reduces DC differentiation from monocyte precursors. *Eur J Immunol* 31, 1602-16092001
72. Hilf N, H Singh-Jasuja, P Schwarzmaier, C Gouttefangeas, HG Rammensee, H Schild: Human platelets express heat shock protein receptors and regulate dendritic cell maturation. *Blood* 99, 3676-3682 (2002)
73. Vegh Z, A Mazumder: Generation of tumor cell lysate-loaded dendritic cells preprogrammed for IL-12 production and augmented T cell response. *Cancer Immunol Immunother* 52, 67-79 (2003)
74. Schnurr M, P Galambos, C Scholz, F Then, M Dauer, S Endres, A Eigler: Tumor cell lysate-pulsed human dendritic cells induce a T-cell response against pancreatic carcinoma cells: an *in vitro* model for the assessment of tumor vaccines. *Cancer Res* 61, 6445-6450 (2001)
75. Lewalle P, R Rouas, F Lehmann, P Martiat: Freezing of dendritic cells, generated from cryopreserved leukaphereses, does not influence their ability to induce antigen-specific immune responses or functionally react to maturation stimuli. *J Immunol Methods* 240, 69-78 (2000)
76. Westermann J, IJ Korner, J Kopp, S Kurz, M Zenke, B Dorken, A Pezzutto: Cryopreservation of mature monocyte-derived human dendritic cells for vaccination: influence on phenotype and functional properties. *Cancer Immunol Immunother*, 52:194-198 (2003)
77. John J, J Hutchinson, A Dalgleish, H Pandha: Cryopreservation of immature monocyte-derived dendritic cells results in enhanced cell maturation but reduced endocytic activity and efficiency of adenoviral transduction. *J Immunol Methods* 272, 35-48 (2003)
78. Morse MA, RE Coleman, G Akabani, N Niehaus, D Coleman, HK Lyerly: Migration of human dendritic cells after injection in patients with metastatic malignancies. *Cancer Res* 59, 56-58 (1999)
79. Fong L, D Brockstedt, C Benike, L Wu, EG Engleman: Dendritic cells injected via different routes induce immunity in cancer patients. *J Immunol* 166, 4254-4259 (2001)
80. Ribas A, LH Butterfield, B Hu, VB Dissette, WS Meng, A Koh, KJ Andrews, M Lee, SN Amar, JA Glaspy, WH McBride, JS Economou: Immune deviation and Fas-mediated deletion limit antitumor activity after multiple dendritic cell vaccinations in mice. *Cancer Res* 60, 2218-2224 (2000)
81. Kim KD, HG Lee, JK Kim, SN Park, IS Choe, YK Choe, SJ Kim, E Lee, JS Lim: Enhanced antigen-presenting activity and tumour necrosis factor-alpha-independent activation of dendritic cells following treatment with Mycobacterium bovis bacillus Calmette-Guerin. *Immunology* 97, 626-633 (1999)
82. Stuber E, W Strober, M Neurath: Blocking the CD40L-CD40 interaction *in vivo* specifically prevents the priming of T helper 1 cells through the inhibition of interleukin 12 secretion. *J Exp Med* 183, 693-698 (1996)
83. Cella M, D Scheidegger, K Palmer-Lehmann, P Lane, A Lanzavecchia, G Alber: Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J Exp Med* 184, 747-752 (1996)
84. Waller EK, MS Ernstoff: Modulation of antitumor immune responses by hematopoietic cytokines. *Cancer* 97, 1797-1809 (2003)
85. Rubinstein MP, AN Kadima, ML Salem, CL Nguyen, WE Gillanders, DJ Cole: Systemic administration of IL-15 augments the antigen-specific primary CD8+ T cell response following vaccination with peptide-pulsed dendritic cells. *J Immunol* 169, 4928-4935 (2002)
86. Zitvogel L, B Couderc, JI Mayordomo, PD Robbins, MT Lotze, WJ Storkus: IL-12-engineered dendritic cells serve as effective tumor vaccine adjuvants *in vivo*. *Ann New York Acad Sci* 795, 284-293 (1996)

87. Moingeon P: Strategies for designing vaccines eliciting Th1 responses in humans. *J Biotech* 98, 189-198 (2002)
88. Tuettenberg A, H Jonuleit, T Tuting, J Bruck, J Knop, AH Enk: Priming of T cells with Ad-transduced DC followed by expansion with peptide-pulsed DC significantly enhances the induction of tumor-specific CD8+ T cells: implications for an efficient vaccination strategy. *Gene Ther* 10, 243-250 (2003)
89. Lau R, F Wang, G Jeffery, V Marty, J Kuniyoshi, E Bade, ME Ryback, J Weber: Phase I trial of intravenous peptide-pulsed dendritic cells in patients with metastatic melanoma. *J Immunother* 24, 66-78 (2001)
90. Banchereau J, AK Palucka, M Dhodapkar, S Burkeholder, N Taquet, A Rolland, S Taquet, S Coquery, KM Wittkowski, N Bhardwaj,L Pineiro, R Steinman, J Fay: Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. *Cancer Res* 61, 6451-6458 (2001)
91. Krause SW, C Neumann, A Soruri, S Mayer, JH Peters, R Andreesen: The treatment of patients with disseminated malignant melanoma by vaccination with autologous cell hybrids of tumor cells and dendritic cells. *J Immunother* 25, 421-428 (2002)
92. Smithers M, K O'Connell, S MacFadyen, M Chambers, K Greenwood, A Boyce, I Abdul-Jabbar, K Barker, K Grimmett, E Walpole, RThomas: Clinical response after intradermal immature dendritic cell vaccination in metastatic melanoma is associated with immune response to particulate antigen. *Cancer Immunol Immunother* 52, 41-52 (2003)
93. Hajek R, AW Butch: Dendritic cell biology and the application of dendritic cells to immunotherapy of multiple myeloma. *Med Oncol* 17, 2-15 (2000)
94. Titzer S, O Christensen, O Manzke, H Tesch, J Wolf, B Emmerich, C Carsten, V Diehl, H Bohlen: Vaccination of multiple myeloma patients with idiotype-pulsed dendritic cells: Immunological and clinical aspects. *Brit J Hematol* 108, 805-816 (2000)
95. Liso A, KE Stockerl-Goldstein, S Auffermann-Gretzinger, CJ Benike, V Reichardt, A van Beckhoven, R Rajapaksa, EG Engleman, KG Blume, R Levy: Idiotype vaccination using dendritic cells after autologous peripheral blood progenitor cell transplantation for multiple myeloma. *Biol Blood Marrow Transplant* 6, 621-627 (2000)
96. Su Z, J Dannull, A Heiser, D Yancey, S Pruitt, J Madden, D Coleman, D Niedzwiecki, E Gilboa, J Vieweg: Immunological and clinical responses in metastatic renal cancer patients vaccinated with tumor RNA-transfected dendritic cells. *Cancer Res* 63, 2127-2133 (2003)
97. Munoz N, FX Bosch, S de Sanjose, R Herrero, X Castellsague, KV Shah, PJ Snijders, CJ Meijer, Int Agency for Res on Cancer Multicenter Cervical Cancer Study G: Epidemiologic classification of human papillomavirus types associated with cervical cancer. *New Engl J Med* 348, 518-527 (2003)
98. Koutsky LA, KA Ault, CM Wheeler, DR Brown, E Barr, FB Alvarez, LM Chiacchierini, KU Jansen, Proof of Principle Study I: A controlled trial of a human papillomavirus type 16 vaccine. *New Engl J Med* 347, 1645-1651 (2002)
99. Rudolf MP, SC Fausch, DM Da Silva, WM Kast: Human dendritic cells are activated by chimeric human papillomavirus type-16 virus-like particles and induce epitope-specific human T cell responses *in vitro*. *J Immunol* 166, 5917-5924 (2001)
100. Santin AD, PL Hermonat, A Ravaggi, S Bellone, JJ Roman, S Jayaprabhu, S Pecorelli, GP Parham, MJ Cannon: Expression of CD56 by human papillomavirus E7-specific CD8+ cytotoxic T lymphocytes correlates with increased intracellular perforin expression and enhanced cytotoxicity against HLA-A2-matched cervical tumor cells. *Clin Cancer Res* 7, 804s-810s (2001)
101. Santin AD, S Bellone, M Palmieri, B Bossini, JJ Roman, MJ Cannon, E Bignotti, S Cane, S Pecorelli: Induction of tumor-specific cytotoxicity in tumor infiltrating lymphocytes by HPV16 and HPV18 E7-pulsed autologous dendritic cells in patients with cancer of the uterine cervix. *Gynecol Oncol* 89, 271-280 (2003)
102. Hernando JJ, TW Park, K Kubler, R Offergeld, H Schlebusch, T Bauknecht: Vaccination with autologous tumour antigen-pulsed dendritic cells in advanced gynaecological malignancies: Clinical and Immunological evaluation of a phase I trial. *Cancer Immunol Immunother* 51, 45-52 (2002).
103. Kono K, A Takahashi, H Sugai, H Fujii, AR Choudhury, R Kiessling, Y Matsumoto: Dendritic cells pulsed with HER-2/neu-derived peptides can induce specific T-cell responses in patients with gastric cancer. *Clin Cancer Res* 8, 3394-3400 (2002)
104. Yu JS, CJ Wheeler, PM Zeltzer, H Ying, DN Finger, PK Lee, WH Yong, F Incardona, RC Thompson, MS Riedinger, W Zhang, RM Prins, KL Black: Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res* 61, 842-847 (2001)
105. Brossart P, S Wirths, G Stuhler, VL Reichardt, L Kanz, W Brugger: Induction of cytotoxic T-lymphocyte responses *in vivo* after vaccinations with peptide-pulsed dendritic cells. *Blood* 96, 3102-3108 (2000).
106. Stift A, J Friedl, P Dubsky, T Bachleitner-Hofmann, G Schueller, T Zontsich, T Benkoe, K Radelbauer, C Brostjan, R Jakesz, M Gnant: Dendritic cell-based vaccination in solid cancer. *J Clin Oncol* 21, 135-142 (2003).

Key Words: Treatment, Therapeutics, Neoplasia, Tumor, Vaccine, cancer, immunotherapy, immunity, T cell, CTL, Tumor-Associated Antigen, Review

Send correspondence to: Michael A. Morse, M.D. Department of Surgery, Lehigh Valley Hospital, 1240 S. Cedar Crest Blvd.Allentown, PA 18103, Tel: 919-684-6408, Fax: 919-681-7970, E-mail: morse004@mc.duke.edu

http://www.bioscience.org/current/vol12.htm