Dendritic cell vaccines

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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.
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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 cytokines, such as GM-CSF, granulocyte-colony stimulating factor (G-CSF), Flt3 ligand (Flt3L) and progeniploietins (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^hi 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).

3.2. 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 progeniploietins (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...
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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 unfractonated 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
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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, Schmurr 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 elborate 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 administration 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
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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 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 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.
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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 idiotypic 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-idiotypic 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 (PB-PCT) 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. Reicheardt and colleagues treated twelve multiple myeloma patients with high-dose therapy and peripheral blood stem cell transplantation (PB-PCT) 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
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lysateloaded 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/nu overexpressing gastric cancers were immunized intradermally with DC pulsed with HER-2/neu (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 use 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 51Cr-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 IFNgamma 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.

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