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Review

Dendritic Cell Vaccines in the Treatment of Multiple Myeloma

Advances and Limitations

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Abstract

Dendritic cells (DCs) are antigen-presenting cells that play a key role in the induction of cytotoxic T-lymphocytes. Adjuvant immunotherapy with antigen-loaded DCs represents an attractive anticancer strategy for multiple myeloma (MM). Autologous DCs loaded with idiotypic protein or other myeloma-associated antigen have been used in several clinical trials. Preclinical and first clinical experience have provided valuable insights in the mechanisms of cellular immunity, but few, if any, patients with MM benefited from such vaccination. Taken together, the data suggest that antitumor T-cell responses fail in MM because of a deregulated cytokine network, downregulation of costimulatory surface receptor expression, and changes in T-cell repertoire, enabling tumor cells to escape immune effectors by preventing the antitumor immune response. We discuss current clinical protocols for DC-based immunotherapy in MM and review some strategies that may increase the efficacy of DC vaccines.

Key Words: Dendritic cells; multiple myeloma; immunotherapy; cytotoxic T-cells; tumor immunology.

Cell Immunotherapy for Multiple Myeloma

Multiple myeloma (MM) is a disease caused by clonal proliferation of a malignant plasma cell clone. Its incidence is 4 in 100,000, constituting about 1% of all cancers. In most patients, it follows a slowly progressive course with a median survival of 3–5 yr if treated by chemotherapy with or without autologous stem cell transplantation and is considered incurable by currently available therapies *(1).* There are several reasons why MM seems particularly suitable for cell immunotherapy. Significant antimyeloma immune responses leading to durable clinical remissions are seen in the setting of allogeneic nonmyeloablative stem cell transplantation and/or donor lymphocyte infusion, suggesting the presence of strong antigens on malignant plasma cells *(2).* The malignant clone of plasma cells specifically expresses a monoclonal immunoglobulin, called idiotypic (Id) protein, which can be readily isolated from the plasma of MM patients *(3,4).* It has been proven that in vitro induced Id-specific cytotoxic T-lymphocytes are able to lyse autologous malignant cells, making it an attractive target antigen for specific immunization *(5).*

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However, vaccination strategies that attempted to elicit immune cell responses to malignant plasma cell-associated antigens using the naked Id protein, or Id protein conjugated with immunogenic carriers such as keyhole limpet hemocyanin (KLH) or the human granulocyte and macrophage colony-stimulating factor (GM-CSF) met with limited success *(4,6)*. The use of autologous dendritic cells (DCs) loaded with a tumor-associated antigen was suggested in an effort to induce more efficient specific antitumor cytotoxic T-lymphocytes (CTL) production *(7)*.

Dendritic cells play a central role in most current experimental protocols that attempt to elicit a specific anticancer immune response. They appear critical for the induction and maintenance of CTLs *(8)*. Furthermore, data indicate that cytokine-induced killer cells also react against myeloma cells following exposure to Id-pulsed DCs *(9)*. In some animal models, the antitumor protection following vaccination with antigen-loaded DCs was found to be T-cell-independent, implicating that specific antibodies also play a role in the DC-mediated immune responses *(10)*.

Dendritic cells differ functionally and phenotypically, depending on their stage of activation *(11)*. Immature DCs are extremely efficient at capturing and processing antigens *(12),* whereas maturation of DCs induced by inflammatory mediators results in reduced capacity to endocytose antigens and increased levels of adhesion and costimulatory molecules needed for optimal major histocompatibility complex (MHC)-dependent presentation of peptides to CTLs *(13–15)*. They express large amounts of MHC class II molecules and lack the lineage markers such as CD14 (monocytes/macrophages); CD3 (T-cells); CD19, CD20, and CD24 (B-cells); CD56 (NK cells); and CD66b (granulocytes). CD1a is preferentially expressed on human immature myeloid DC, whereas CD83 is found on mature DCs, which also express high levels of costimulatory molecules (CD80, CD86, CD40) and adhesion molecules, including CD11a, CD11c, CD50, CD54, CD58, and CD102 *(16)*.

Large quantities of DCs presenting a tumor-associated antigen (typically more than 1×10^6 cells) are needed for clinical applications. A number of protocols for DC culture and ex vivo expansion have been described, using various sources of DC precursors such as bone marrow *(17,18),* cord blood *(19),* peripheral blood mononuclear cells *(20,21),* peripheral blood stem cells *(22),* and CD14+ monocytes *(23)*.

The pilot trial using antigen-pulsed DCs in the treatment of a lymphoid malignancy was performed by Levy and colleagues in 1996 *(24,25)*. The successful vaccination of four patients with follicular non-Hodgkin's lymphoma with autologous DCs opened the door to antiidiotypic vaccination for MM.

Clinical Trials

The currently available clinical data on the efficacy of DC vaccines in MM come from several groups that have already published their experience in full or preliminary reports *(26–34)*.

Scientists from Stanford University have performed the as-yet largest clinical study involving the vaccination of MM patients with antigen-loaded DCs *(26,27)*. They vaccinated 26 patients 9–12 mo after high-dose chemotherapy with autologous peripheral stem cell transplantation. The patients were given only one dose of DCs loaded either with Id protein or with a conjugate of Id protein with KLH. Several subcutaneous boosts of the Id–KLH conjugate followed at 4-wk intervals. In 24 of 26 patients who received at least two booster vaccinations, a KLH-specific proliferative response was induced, contrasting with only 4 patients who developed an Id-specific proliferative response as detected by in vitro thymidin proliferation assay *(26,27)*. This result indicates rather poor immunogenicity of Id protein.

British researchers from Cardiff vaccinated six MM patients with Id-loaded DCs, which were administered intravenously three times. In one patient, a decrease in the serum level of Id protein by 25% was detected. Id-specific proliferative response of mononuclear cells was induced in five of six patients *(28,29)*.

Cull and colleagues treated two patients with advanced MM with a series of four vaccinations using autologous Id-protein-pulsed DCs combined with adjuvant GM-CSF administered immediately after each DC infusion. T-Cell proliferative response developed in both and anti-Id IgM antibodies in one patient. No Id-specific cytotoxic T-cell response could be demonstrated *(30)*.

Titzer et al. reported on a study that included 11 patients with MM *(31)*. Their vaccination strategy was based on the administration of one dose of Id-loaded DCs followed by three doses of either Id-loaded DCs or three doses of a hybrid protein consisting of the autologous Id protein joined with GM-CSF. Increased levels of anti-Id antibodies were detected in 3 of 10 patients. In 4 of 10 patients, Id-specific T-cell response was demonstrated using ELISPOT analysis. Most importantly, in one patient, a decrease in bone marrow infiltration by plasma cells was found, a first evidence of in vivo cytolytic activity of immune mechanisms activated by autologous antigen-loaded DCs *(31)*.

The vaccine was well tolerated in all described clinical trials, producing only mild toxicity, including erythema, induration, and soreness at the injection site, low-grade fever, and an isolated case of postinfusion thrombophlebitis *(26–34)*.

Preliminary reports by other groups working in the field have been published and more results are awaited in the hope of finding the best vaccination design that would maximize the specific immune response. Significant numbers of patients were recruited for these recent clinical trials, but only modest clinical and immunological responses to vaccination were detected *(32–34)*. Despite these rather disappointing results, DC vaccines have already become commercially available for use in MM. A US-based company has administered them to more than 75 patients *(35)*.

Problems and Limitation of Dendritic Cell Vaccines for Multiple Myeloma

There are only few, if any, patients with MM who responded clinically to vaccination with antigen-loaded autologous DCs. There may be several reasons for this failure. No single, strong tumor-associated antigen suitable for peptide vaccination, analogous to MAGE peptides in melanoma *(36)* or Her2 Neu in breast cancer *(37),* has been identified for MM. Several studies point to an inherent immune system dysregulation that complicates the development of immunotherapeutic strategies for MM. MM is associated with many defects in the host immune system *(38,39)*. Functional changes occur in T-cells, B-cells, macrophages, and NK/LAK cells *(38)*. There are many immunosuppressive cytokines such as tumor growth factor-β and interleukin-10 in the tumor microenvironment and in vivo priming of T-cells might be impaired or completely abolished *(38)*. Patients with MM have altered distribution of CD4 and CD8 lymphoid subsets with a reduction of CD4+ cells, particularly the naive CD4+/CD45RA+ cells. This decrease is more pronounced in patients with advanced disease *(38)*. Thus, antitumor T-cell responses fail in MM as a result of a deregulated cytokine network, defects in costimulatory surface receptors, and changes in T-cell repertoire enabling tumor cells to escape immune surveillance by preventing the antitumor Th1 immune response *(38,40,41)*.

A controversy surrounds the question of whether DCs derived from patients with multiple myeloma are fully able to stimulate specific CTL response *(42,43)*. They apparently fail to upregulate the expression of important costimulatory surface molecules, such as CD80, which provide key signals for the activation of resting CTLs and the prevention of anergy *(43)*.

How to Enhance the Efficacy of Dendritic Cell Vaccines

In spite of the above-described problems, there is a hope that new advances in antigen delivery, changes in vaccination route and timing, and/or addition of adjuvants will provide us with a new therapeutic tool useful for some patients with MM.

Several strategies have proved useful in correcting or circumventing the abnormalities in immune functions seen in the cancer patient. The defects in costimulation may be repaired using viral vectors to introduce DNA encoding for CD80 and other costimulatory molecules into DCs in an effort to stimulate specific T-cell proliferation, cytokine release, and induction of cytolytic activity against malignant cells *(44)*. Cytokines known to enhance cell-mediated immune responses such as GM-CSF or interleukin-2 may be administered as adjuvants with the vaccines aiming to create an environment where specific immune responses are readily induced *(45)*.

New, stronger antigens for clinical use are searched. Schultze coined the term *tumor-rejection antigens* for antigens that are not only selectively associated with cancer but can also be efficiently targeted to

destroy tumor cells, leading to clinically significant tumor regression *(46).* Telomerase catalytic subunit (hTERT), a tumor-associated antigen expressed in MM as well as in other malignancies, is capable of triggering antitumor CTL responses. Its usefulness as a target for vaccination is under investigation *(47).* Some authors have suggested the use of whole cells rather than a single tumor-associated antigen. Myeloma cell lysate could be used to load DCs in a revival of first in vitro experimental designs *(48).* In a different approach, Anderson et al. discovered that myeloma cells can be fused with autologous DCs in the presence of polyethylenglycol and that the resulting hybrid cell efficiently stimulates CTLs from patients with MM *(47).*

The route of DC administration may be an important factor for the development of immune responses. Whereas intravenous DCs stimulate antibody production via the Th2 cascade, intradermal, subcutaneous, and intralymphatic injections induce predominantly Th1-directed immunity with detectable interferon (IFN)-γ production promoting CTL expansion *(16,49,50).* Coming from experience with prostate cancer *(49),* this knowledge needs to be reflected in experimental protocols for the clinic.

The design of vaccination studies should also be reassessed. So far, antimyeloma vaccines have been given mostly to patients with advanced disease *(28–30)* or those after high-dose chemotherapy with autologous stem cell transplantation *(26,27).* Such a setting makes it very difficult to evaluate the clinical response to vaccination, and future clinical trials should concentrate on patients with stable, detectable disease. In one study, the T-cell repertoire of MM patients was severely disrupted for as long as 13 mo after stem cell transplantation compared with age-matched normal donors. This may affect the clinical outcome of vaccine-based strategies delivered at the stage of minimal residual disease *(51).*

Conclusions

Dendritic cell vaccination has not yet caused a breakthrough in immunotherapy for MM. However, preclinical and first clinical experience have provided valuable insights in the mechanisms of cellular immunity that will undoubtedly provide a basis for a new generation of clinical protocols. Taken together, the data suggest that antitumor T-cell responses fail in MM as a result of a deregulated cytokine network, defects in constimulatory surface receptor expression, and changes in T-cell repertoire enabling tumor cells to escape immune effectors by preventing the antitumor Th1 immune response. A better understanding of the dynamics of antitumor response in vivo in MM patients is another important issue that needs to be addressed in the near future. Immune competence of a patient should be evaluated before and during immunotherapy, as its acquired defects could be a key underlying factor for an insufficient immune response to vaccination, especially in MM and other lymphoid malignancies. Such studies will hopefully help us improve antimyeloma vaccines in the future. In addition, methods for specific antitumor CTL production and expansion are rapidly developing *(52)* and may soon be available for applications in MM and other cancers, obviating the need to administer antigen-presenting cells and enhancing the efficacy of immunotherapeutic protocols.

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