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Production of myeloid dendritic cells (DC) pulsed with tumor-specific idiotype protein for vaccination of patients with multiple myeloma

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Background

Immunotherapy of cancer with DC vaccines has produced encouraging results in clinical trials. Antigen (Ag)-pulsed DC have elicited $CD4^+$ and $CD8^+$ T-cell immunity and tumor regression in humans. However, there is no standard method of DC production. The DC phenotype, number and Ag-loading process used in these studies have varied, making comparisons between trials difficult.

Methods

In the present report a reproducible method was developed for the production of a DC-based vaccine. Monocytes were enriched by adhesion from healthy donor apheresis products and cultured with growth factors for maturation into DC. The cells were loaded with the tumor Ag idiotype proteins from patients with multiple myeloma. DC culture and Ag loading were performed in an automated and closed system. The DC product was characterized for phenotype by flow cytometry and for function in Ag uptake and Ag presentation.

Results

These monocyte-derived DC expressed high levels of costimulatory molecules (CD80/86). Ag-pulsed DC functioned to induce allogeneic proliferative lymphocyte responses and Ag-specific cytotoxic T lymphocyte (CTL) responses. The DC viability, phenotype and function were well preserved following prolonged frozen storage. Aliquots from the product of a single DC preparation could be used for sequential vaccinations without batch to batch variability.

Discussion

Ag-pulsed DC can be reproducibly generated for clinical use. These standardized methods are now being employed for a clinical trial to evaluate idiotype-pulsed DC vaccine therapy following non-myeloablative transplant for the treatment of multiple myeloma.

Keywords

APC, DC, idiotype, multiple myeloma, T cell.

Introduction

DC were initially described by their adherent properties and unique morphology in the early 1970s [1]. DC are now considered nature's adjuvant and the sentinels of the immune system [2]. DC, although a rare component of peripheral blood [3], are particularly potent in processing and presenting Ag to the immune system. Following Ag uptake and processing, DC found in the peripheral circulation or tissue can then migrate to draining lymph nodes for Ag presentation. For an efficient T-lymphocyte response, a mature DC phenotype with surface expression of costimulatory molecules and major histocompatibility complex (MHC) Ag is needed. These changes occur after immature DC encounter pathogens that trigger DC maturation and migration to secondary lymphoid organs.

Because of these unique properties, DC are a powerful tool to manipulate the immune system and may be useful for cancer immunotherapy [2]. The first successful clinical study using DC was performed in the 1990s for the treatment of B-cell lymphoma [4]. This study used peripheral blood DC, isolated by large-scale leukophoresis and density gradients. Unfortunately, peripheral DC represent $<1-2\%$ of the peripheral blood mononuclear cells (PBMC) and they are difficult to expand and mature in culture. DC can also be isolated from peripheral blood using monocloral antibodies (Mab) [5]. Other researchers have evaluated recombinant Ag-loaded peripheral blood-

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derived DC as a therapeutic vaccine for prostate cancer [6]. Several phase I/II trials have demonstrated safety and tolerability of this vaccine approach. Clinical effects have been shown using this immunotherapy approach and have prompted larger definitive trials [7]. The studies required multiple leukapheresis to isolate blood-derived autologous DC and an overall low yield of cellular product. Human DC can be generated *in vitro* from peripheral blood from $CD14^+$ monocytes or $CD34^+$ progenitors. The usual method of isolating human DC uses plastic adherence in flasks to select monocytes from PBMC [8–10]. The adherent monocytes are cultured with granulocyte macrophage-colony stimulating factor (GM-CSF) and interleukin-4 (IL-4). Newer methods have incorporated immunomagnetic cell sorting to enrich monocytes from leukapheresis samples [11,12]. There are now data suggesting that DC isolated by positive selection of monocytes using anti-CD14-coated microbeads may be defective in cytokine production [13]. In contrast, DC generated by adherence methods are not defective in cytokine production and function well to generate peptide-specific $CD8^+$ T cells [14]. Moreover, DC loaded with tumor-derived Ag mRNA generated potent antigen presenting cells (APC) [15]. DC transfected with mRNA encoding tumor-associated Ag have resulted in the generation of tumor-Ag specific cytotoxic T cells [16,17]. More recently, direct injection of tumor-associated Ag encoding mRNA have generated anti-tumor T-cell responses [18] but were found to be inefficient at overcoming tolerance to tumor/self-Ag in transgenic mouse models. Attempts at optimizing Ag presentation using transfection methods with RNA continue to be investigated [19,20]. Viral transduction of DC *in vitro* [21] is also under investigation. Unfortunately, these approaches increase the chances of contamination and require gene delivery by virus. All of the open culture methods are laborious, have a low yield of DC and a substantial risk of contamination. Attempts to shorten the DC culture duration may decrease the risk of contamination [22–24]; however, additional *in vitro* and clinical investigations will be needed to demonstrate that the '48 h DC differentiation is equivalent to more standard DC 7–8 day DC maturation approaches.

Closed system approaches have been developed using immunomagnetic bead enrichment [25,26] and have generated DC equivalent to DC generated using conventional flask methodology [26]. In the current study, we describe methods to produce a DC vaccine product derived from normal donors for use in conjunction with allogeneic BM transplantation for the treatment of multiple myeloma. In this method monocytes are enriched by adherence from leukapheresis products from healthy donors. Large-scale production of DC is performed in the presence of IL-4 and GM-CSF in a closed culture system, the AastromReplicell™ Cell Production System. The immunoglobulin (Ig) produced by the myeloma tumor is purified from the serum of the patient and chemically conjugated to keyhole limpet hemocyanin (KLH) protein. This coupled protein is delivered into the closed culture system and the DC are allowed to mature under the influence of tumor necrosis factor α (TNF-α).

The methods described here were validated with myeloma proteins from multiple different patients and on monocytes derived from multiple different normal donors. Each Ig molecule produced by the plasma cell has a unique variable region sequence, termed idiotype (Id), which can be recognized as an Ag. DC pulsed with tumor-specific Id protein Ag and used for clinical vaccination of other B-cell malignancies has been successful in generating immune responses [4,27]. Autologous Id-pulsed DC have been shown to be feasible and safe in the treatment of multiple myeloma [28]. A subsequent clinical trial demonstrated anti-Id responses in patients treated post-autologous transplant with Id-pulsed DC vaccines [29].

Non-myeloablative stem cell transplantation has improved outcomes in multiple myeloma because it produces a graft-versus-myeloma effect. Despite this overall benefit, disease relapse still occurs at a high rate. Therefore, a vaccine approach with Ag-loaded DC (Figure 1), as outlined here, may provide additional benefit for treatment of this disease.

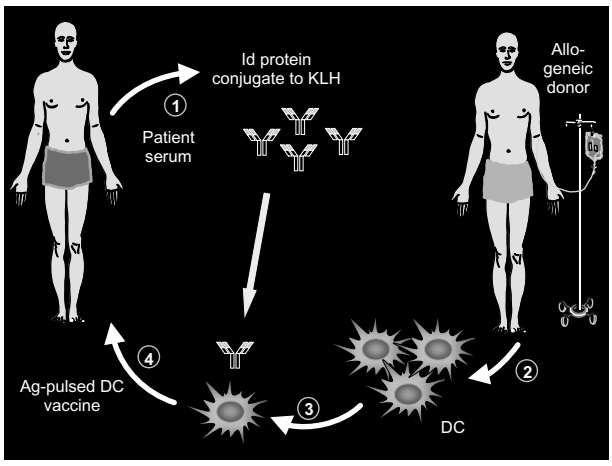

Figure 1. *Schema of DC vaccine production and patient vaccination.*