ORIGINAL REPORT

Vaccination of Diffuse Large B- Cell Lymphoma Patients with Antigen-Primed Dendritic Cells

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Abstract- Dendritic cells (DCs) are professional antigen presenting cells that have a potential role in the initiating of immune responses. The cell vaccination is a new strategy in treatment of infectious diseases and cancers. In this study, we have generated monocyte-derived dendritic cells of lymphoma patient's peripheral blood mononuclear cells then; these cells were used as vaccine in lymphoma patients. We generated dendritic cell vaccine from lymphoma patient's blood monocytes with human interleukin-4, granulocyte monocyte colony stimulating factor and then, antigen–primed Dcs were administrated subcutaneously close to the inguinal lymph nodes after maturation of dendritic cells. After 7 days, we analyzed immune response in lymphoma patients with determining of LDH, Beta 2 Microglobulin, CD4+T cell percent, CD8+ Tcell percent and Tumor size before and after vaccination. Furthermore, phenotypic and functional analysis of dendritic cells was performed using anti CD83-FITC monoclonal antibodies. Before vaccination, the mean ± SD of LDH was 530.62±140.65 but after vaccination it was 459±109.45 that significantly different between experimental groups (P=0.002). In addition, the CD8+ T cells percentage significantly different between two groups (P=0.002). We concluded that the use of dendritic cell probably is one of the suitable noninvasive treatments for lymphoma patients that they have not response to chemical drugs.

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Introduction

It is well known that dendritic cells (DCs) have a critical role in antigen presentation (1). DCs are professional antigen presenting cells with a variety of positive as well as negative regulatory signals. Dendritic cell maturation can be triggered by antigen uptake and cytokines. DCs, after uptake of antigens move to adjacent lymph nodes and present antigens to T cells (2). In this way, maturation markers such as CD83 up regulated on the dendritic cells (3). DCs can be generated by peripheral monocytes in the presence of interleukin-4 and GM-CSF in vitro (4). Then, these cells can be matured in the culture media with tumor necrosis factor- α (TNF-α) or the others maturation factors (5). Dendritic cell vaccine is a new strategy in treatment of infectious diseases and cancers (6). Up to now, many experiments conducted to evaluate the role of dendritic cells on the malignant diseases in vivo and in vitro (7). Diffuse large B- cell Lymphoma is a lymphoproliferative disorder that usually manifested by lymphadenopathy and anemia (8). Tumor antigens in lymphoma patients are weak immunogens, thus can not suitably stimulate immune responses (9). Tumor-associated antigens in the cancerous patients are the most candidate for stimulating of specific T cells (10). The studies showed that, antigen–primed Dcs can be used in malignant melanoma and breast cancer (11). Cancer immunotherapy with monocyte-derived dendritic cells is a new treatment model for spontaneous remission conditions (12).

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In this study, we have evaluated the effect of autologous monocyte– derived dendritic cell vaccine in the diffuse large B-cell lymphoma patients after generation of dendritic cell from peripheral blood monocytes.

Materials and Methods

Patients and antigenic suspension

The patients were selected from the hematology and oncology center of the university hospital and admitted to Tooba Medical Diagnosis Laboratory (Sari, Iran). The study was conducted on a group of 8 patients with diffuse large B-cell lymphoma. The peripheral blood samples were collected after consent confirm of the volunteer patients. The lymph node tumors from each patient were isolated and tumor cell lysates were disrupted by two cycles of freeze and thaw in liquid nitrogen and 37º C water bath. Then, after centrifugation for removal of particles, the supernatant were passed through filter (0.2µm). The protein concentrations in supernatants were determined by with Bradford method (13).

Dendritic cell generation

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by centrifugation on a Ficoll Histipaque 1.077 (Lymphoprep, Norway). Cells from interphases were collected and washed three times with RPMI-1640 medium (Gibco, USA). Cells were counted and cell viability was determined by trypan blue exclusion.

$CD14^+$ monocytes (5×10^5) were cultured in RPMI-1640 medium supplemented with 10% patient serum, 100units/ml penicillin and 100µg/ml streptomycin for 5 days with different combinations of purified recombinant human cytokines: 10 ng/ml human interleukin-4 (hIL-4, bender med Germany), and 10 ng/ml human granulocyte macrophage colony stimulating factor (hGM-CSF, bender med. Germany) to generate immature DC. To induce maturation of dendritic cells, the immature DCs were further incubated with 10ng/ml tumor necrosis factor alfa (TNF-α, bender med. Germany) and 10ng/ml antigenic suspension for additional 48h before harvest.

Flowcytometry

After 7 days, dendritic cells were stained for 30 min at 4°C with the antiCD83-FITC (Dako, Germany) or isotype control antibodies (Dako, Germany). Surface expressions of the antigens were measured by flowcytometry (partec pas, Germany).

Injection of dendritic cell vaccine to lymphoma patients

Antigen-primed Dcs were administrated subcutaneously close to the inguinal lymph nodes after maturation of dendritic cells. $3\text{-}10\times10^6$ DCs in physiologic serum were injected per vaccine in two steps. After injection of antigen-primed DCs, the patients were proctored for 24h for seeing any side effects. One week after the vaccination, we assessed lactate dehydrogenate (LDH), β2 microglobulin (β2M), $CD4^+$Tcell, $CD8^+$T cell and Size of tumor in comparison before vaccine injection.

Co-culture of DC with $CD4^+$ T cells

Before vaccine injection, 1×10^5 $CD4^+$ T cells and autogenic 1×10^4 monocyte-derived DCs from 8 different patients were cultured. All samples were run in triplicates. The cells were incubated at 37°C in a humidified 5% CO_2 atmosphere for 5 days. Proliferate T cells responses to monocyte-derived DC were measured by adding 200 µl 3-[4,5-dimethylthiazolyl]-2,5-diphenyl-tetrazolium bromide (MTT; Sigma) for 4h and reading color changes at 575 nm wave length representing cell proliferation (14).

Statistical analysis

For statistical analysis, we used paired *t*-tests. The *P* values were determined in all cases and $P<0.05$ was considered as statistically significant.

Results

Biomarkers concentration in Lymphoma patients before and after vaccination

The mean age of the lymphoma patients was 45.37 (7Male, 1 Female), with an age range 17-59year. One week after the vaccination, we assessed lactate dehydrogenate (LDH), β2 microglobulin (β2M), $CD4^+$Tcell, $CD8^+$T cell and Size of tumor in comparison with before vaccine injection. Before vaccination, the Mean ± SD of serum LDH was 459±109.45 and after vaccination, it was 530.62±140.65 that was statistically significant ($P=0.002$). There was not a significant difference between serum β2 microglubin concentrations between two times in the patients ($P=0.123$).

Dendritic cell phenotype

Mature DCs generated from blood mononuclear cells with GM-CSF and IL-4 in the presence of tumor

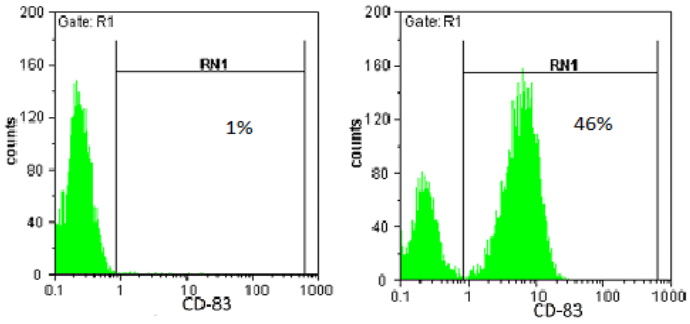

Figure 1. Flowcytometry Analysis showed the minimal expression level of CD83 on their surface before stimulation with antigen and TNF (Immature Dc). Antigen significantly acted by up regulating surface level of CD83 on dendritic cells (Mature DC) .Expression of the CD83 marker indicated the maturation of DCs. In the corner of each graph, mean percentage of positive cells is written.

Table1. The concentration of LDH and β2microglubolin after and before vaccination.

Vaccination Group	Biomarker	
	LDH	β2m
Before vaccination	530±140.65	4.76±2.56
After vaccination	452±102.38	3.87±1.67
P value	0.02	0.123

Values are mean ±SD

antigen followed by TNF-α stimulation were analyzed for the expression of DC surface markers. Analysis of phenotypes showed the expression of CD83 minimal level on their surface before stimulation with the antigen. Antigen significantly up regulated the surface level of CD83 on dendritic cells. Expression of CD83 is indicated of the maturation of dendritic cells (Figure 1). Significantly, $CD8^+$ T cells percent were different before cell vaccination in comparison with after vaccination (*P*=0.002). As shown in table 1, one week after vaccination, the mean of $CD4^+$ T cells percent was 19.64±11.75, with statistically significant difference (*P*=0.016)

Discussion

In this study, we generated dendritic cell vaccine from lymphoma patient's monocytes and then this vaccine was injected to patients after maturation of dendritic cells. In this study, the patients did not receive any anticancer drugs during our study and the efficacy of dendritic cell vaccine tested in culture media with mixed leukocyte reaction before using. DCs are professional antigen presenting cells that have an important role in initiation of immune response (6). Mature dendritic cells have a central regulatory role in T cell response to different antigens. In this way, Immunotherapy is a new strategic method in cancer therapy. Dendritic cell–based immunotherapy can be improved and it can correct anticancer therapy (15). Several studies in murine models showed that use of antigen-treated DCs could eliminate tumor cells (15). These results are corresponding with the data given by Steinmann *et al.* indicating that antigenic suspension can induce a stable maturation of dendritic cell (15).

Table 2. Change of $CD4^+$ and $CD8^+$ percentages after dendritic cell vaccine therapy.

Vaccination Group	T cell	
	$CD4^+$ ± SD	$CD8^+$±SD
Before vaccination	19.64±11.75	6.16±3.33
After vaccination	25.71±16.35	11.81±3.21

The results are mean ± SD

Steinmann *et al.* showed that dendritic cells have an important role in tolerance, autoimmunity and the beginning of immune response (16,17). They have assessed immune responses in cancer therapy after injection of committed cells. The studies showed that antigen-treated DCs could inhibit tumor development with induction of cytotoxic T lymphocyte responses (18). Brossart *et al.* showed that specific cytotoxic T lymphocyte increase after injection of peptide- primed dendritic cells. They used a set of several antigens expressed in breast and ovarian cancer (18). Our results in consistent with this study showed that the CD4 to CD8 ratio decreased after vaccination. Our data indicate significant elevation of $CD4^+$T cells and $CD8^+$ T cells after injection of antigen-treated DCs in comparison with before vaccination (Table 2).

In the present study, tumor size in eight patients decreased after dendritic cell vaccination.

Furthermore, the other studies demonstrated that life styles of cancer patients improved after treatment by cell vaccination. In addition, we showed that LDH as a tumor marker decreased after 7 days of vaccination in comparison with before vaccination (P=0.002). The LDH level in cancer patients is an important criterion after treatment. There are high levels of co-stimulatory molecules, such as CD86, CD80 and HLA-DR on dendritic cell after maturation (16)**.** In this study, the other co-stimulatory molecules on the DCs in addition to CD83 were not detected.

The results show that this method is a beneficial method for resistant patients to anti cancer drugs. It is concluded that antigen-treated DCs vaccine can improve life styles of lymphoma patients and probably, cell vaccination is a new horizon in cancer treatment.

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