*Original Article*

# Decrease of Peripheral Blood CD8+/CD28- Suppressor T Cell Followed by Dentritic Cells Immunomodulation among Metastatic Breast Cancer Patients

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## ABSTRACT

**Objective:** To explore the effects of dentritic cells on the peripheral blood lymphocyte subpopulations of metastatic breast cancer patients treated with chemotherapy.

**Methods:** The current study involved 44 metastatic breast cancer patients treated with docetaxel-based chemotherapy. Among them, 25 cases were treated with dendritic cells derived from $CD34^+$ hematopoietic stem cells enriched autologous peripheral mononuclear cells after chemotherapy, and 19 cases received chemotherapy alone. Peripheral blood samples were collected from each patient before and after treatment, and lymphocyte subpopulations including CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD16+56+, CD3+/CD16+56+, CD4+/CD25+, CD8+/CD28-, CD8+/CD28+, CD4/CD8, DC1, DC2 and DC1/DC2 were analysed by a 3-color flow cytometric analysis.

**Results:** The two treatment groups were well matched with regard to demographic and baseline disease characteristics. Comparing the changes of lymphocyte subpopulations between the two groups, it showed that the difference of the change of CD8+/CD28-lymphocyte had statistic significance. The percentage of CD8+/CD28-lymphocyte was lower in the chemotherapy+DC group, but higher in the chemotherapy-alone group.

**Conclusion:** As CD8+/CD28-lymphocyte represent a kind of suppressive T lymphocyte, we conclude that dentritic cell therapy can relieve immunosuppression to some extent.

**Key words: Metastatic breast cancer; Dentritic cell; Lymphocyte subpopulations; Regulatory T cell**

## INTRODUCTION

The outcome for patients with metastatic breast cancer (MBC) is quite poor. Significant progress has been made in this field. New cytotoxic agents and target therapy have all shown enhanced therapeutic benefit for breast cancer patients[12]. Nonetheless, tumor relapse, the emergence of toxicities, and early death due to the emergence of resistant disease continue to pose great challenges for the clinical management of this disease. Hence, there is an urgent needed for novel intervention strategies capable of synergising with standard adjuvant therapies; maintaining activity against refractory tumors and avoiding further immune suppression. Immunosuppression may contribute to the disease progression. Complex interactions between the immune system and tumor exist in patients with metastatic cancer, nonspecific and systemic immune dysfunction is prevalent in advanced and metastatic cancer[3-5]. In previous studies significant changes have been demonstrated in the numbers and functions of peripheral blood lymphocyte subsets in patients with different malignant disorders[4,6-8]. So to achieve a better outcome for patients with MBC, immunotherapy is one potential treatment option.

Immunotherapeutic strategies that successfully activate the immune system against tumor antigens

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are a prime alternative as they would probably be non-cross-resistant, specifically boost antitumor immunity and have non-overlapping toxicities. Dendritic cells (DCs) are antigen-presenting cells that are capable of stimulating antigen-specific naive and memory T cells. Ample evidences now indicate the existence of an immune response against breast cancer involving DC recruitment and activation within tumor tissue[9,10-12]. Despite the evidences, numerous studies have demonstrated severe phenotypic and functional impairment of DCs in patients with breast cancer[13,14]. Circulating DCs isolated from patients with breast cancer exhibited an impaired capacity to stimulate T lymphocyte proliferation and cytokine secretion[13]. Moreover, patients with metastatic breast cancer showed a large number of immature APCs with poor immunological function in the blood DC compartment[15]. Thus DC therapy is emerging as a crucial strategy to enhance anticancer activity for patients with this disease.

In this study we treated the metastatic breast cancer patients with mature dendritic cell derived from autologous peripheral $CD34^+$ hematopoietic stem cells enriched autologous peripheral mononuclear cells after chemotherapy, and try to explore the effects of dentritic cell on the immune function, such as the peripheral blood lymphocyte subpopulations of these patients.

## MATERIALS AND METHODS

### Patients and Treatment

The current study involved 44 patients with metastatic breast cancer who were admitted to the Department of Medical Oncology, Peking University Cancer Hospital & Institute between January 2008 and May 2009. All these patients were treated with docetaxel-based chemotherapy. Among these patients 25 cases were treated with dendritic cells derived from $CD34^+$ hematopoietic stem cells enriched autologous peripheral mononuclear cells after chemotherapy and 19 cases received chemotherapy alone. As it was a retrospective study, the case number of the two groups was not equal. DCs immunotherapy was approved by Beijing Health Administration and the Local Ethics Committee of Peking University Cancer Hospital & Institute. Written informed consent from each patient was obtained prior to study entry.

### Hematopoietic Stem Cells (HSC) Mobilization and Collection

CD34+ HSCs were mobilized by docetaxel-based chemotherapy and G-CSF. After receiving chemotherapy, when the WBC count of peripherial blood decreased to $\leq 1.5\times10^9$/L, granulocyte colony stimulating factor (G-CSF) at a dose of 5μg/kg/day was given subcutaneously. Autologous peripheral blood mononuclear cells (PBMC) were isolated by leukapheresis when the WBC count increased to $\geq 10.0\times10^9$/L. Leukapheresis was performed by the CBS200 Spectra cell separator and we analyzed the numbers of PBMC and $CD34^+$ HSC in the collections by flow cytometry.

### Culture and Transfusion of Dendritic Cells

DCs were generated by *in vitro* stimulation with IL-4, GM-CSF and TNF-α. Human peripheral blood mononuclear cells (PBMC) were separated by Ficoll , and the adherent cells were grown in X-VIVO 15 (Cambrex Co, USA) with 100 μg/L of GM-CSF (Tebao bio Co., Xiamen China), 10μg/L of IL-4 (R&D Co, USA), and 2.5μg/L of TNF-α (R&D Co, USA). Cell counts and phenotype of DCs were analyzed 10 to14 days after culture. Molecules of CD1a, CD11c, CD80, CD86 and anti-HLA-DR were analyzed by flow cytometer. If the cells differentiated into typical matured dendritic cells with classical phenotype, venous transfusion were conducted immediately. DCs transfusion was conducted every three or four days for altogether 3 times, each time including $10^7$~$10^9$ dendritic cells.

### Blood Samples

Heparinized peripheral blood samples were collected from each patient before chemotherapy. For the DC+chemotherapy group, blood samples were collected again after chemotherapy and the third DC infusion(about four weeks after the chemotherapy) and for the chemotherapy alone group, blood samples were collected again three or four weeks after the chemotherapy or just before the beginning of the next cycle of chemotherapy. All these fresh blood samples were analysed for T cell subpopulations, including CD3+, CD3+/CD4+, CD3+/CD8+, CD3-/CD16+56+, CD3+/CD16+56+, CD4+/CD25+, CD8+/CD28-, CD8+/CD28+, CD4/CD8, DC1, and DC2, DC1/DC2.

### Cell Isolation

Heparinized peripheral blood was obtained, 100μl whole blood was used per tube and they were stained with antibodies, and incubated in the dark at 4°C for 15 min. After hemolysis 10 and gradients Centrifugation, the cells were washed twice in PBS