RESEARCH ARTICLE

Variation of Blood T Lymphocyte Subgroups in Patients with Non- small Cell Lung Cancer

Wen-Jing Wang[1], Zhen Tao[1]*, Wei Gu[2], Li-Hua Sun[2]

Abstract

Objectives: To study variation in T lymphocyte subgoups and its clinical significance in non-small cell lung cancer (NSCLC). Methods: Levels of CD3+ , CD4+ , CD8+ , CD4+/CD8+, NK and Treg cells in peripheral blood of NSCLC cases and healthy adults were determined by flow cytometry. Results: CD3+ , CD4+ and CD4+/CD8+ ratio and NK cells in NSCLCs were decreased significantly in comparison with the control group ($P < 0.01$), and decreased with increase in the clinical stage of NSCLC, while CD8+ cells demonstrated no significant change ($P > 0.05$). Treg cells were significantly more frequent than in the control group ($P < 0.01$), and increased with the clinical stage of NSCLC. Conclusion: The cellular immune function of the NSCLC patients is lowered. It is important to detect change of T lymphocyte subgroups by flow cytometry for the diagnosis, treatment and prognostic assessment of NSCLC patients.

Keywords: Non- small cell lung cancer - lymphocyte subset - flow cytometry - cellular immune

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Introduction

Lung cancer is one of the most common malignant tumors, with high mortality rate worldwide, and still demonstrating a rising trend yearly (Parkin et al., 2005; Liu et al., 2013; Lu et al., 2013). In recent years, research revealed that the development and progression of lung cancer are closely associated with immunological disfunction, especially with T cell function (Nakamura et al., 2000). T cells is mainly composed of CD4+ and CD8+ T cells, maintains the balance of immunological syestm and plays an important role in immunological function. Regulatory T cells (regulatory T cell, Treg) is also a subgroup of T cell, and is reported to bear anti-tumor effect when its level rising (Jason et al., 2003). This study is designed to monitor CD3+, CD4+, CD8+, CD4+/CD8+ and NK cells and Treg, lymphocyte subsubgroup in peripheral blood of patients with non-small cell lung cancer (non-small cell lung cancer, NSCLC) using flow cytometry, and to analyze the relationship between lymphocyte subsubgroup and immune function of patients with NSCLC.

Materials and Methods

Patients

All 153 hospitalized patients with lung cancer who eligible for this study were recuited from June 2009 to October 2012, among them 96 were male, 57 female. Diagnosis of lung cancer in all patients were confirmed pathologically or cytologically. Patients were excluded from this study if they had serious infectious diseases or autoimmune disease. Age of patients was 38-76 years, with an average age of 54.6 years. According to 1997 UICC cancer TNM staging system: 67 patients were stagedI~II, 86 staged III~IV. A group of 50 persons who underwent healthy physical examination was recuited as control.

1.2 sample collection: 2 m l peripheral blood (heparin anticoagulation) from all study subjects was collected at the entrance of the study.

Methods

Experimental reagent and instrument included flow cytometry from Becton Dickinson of the US, lymphocyte subgroup, and Treg detection kit.

T lymphocyte subgroup was tested by using color indicating CD4-FITC/CD8-PE/CD3-PC5, NK cell was tested with color indicating CD3-FITC/CD(15+65)-PE. Heparin anticoagulated peripheral blood 100μL, with 20 μL CD4-FITC/CD8-PE/CD3-PC5 trichromatic labeled antibody, and 20 μL CD3-FITC/CD (15 +65)-PE double color labeled antibody, was put in a light resistant container at room temperature for 15 ~ 20 min. Then Optilyse C RBC cracking fluid was add to the sample for 10 min. Centrifugation was set as 1200 RPM/min for 5 min. PBS washing for three times.Then add 500μL PBS

*[1]Department of Infectious Diseases, [2]Department of Respiration, Nanjing First Hospital, Nanjing Medical University, Nanjing, China *For correspondence: tz1010@126.com*

Table 1. Comparison Between NSCLC and Control Group Regarding Value of T Lymphocyte Subgroup ($\bar{\chi}\pm s$, %)

Groups	Cases	$CD3^+$(%)	$CD4^+$(%)	$CD8^+$(%)	$CD4^+/CD8^+$	NK cell	Treg cell
Control	50	73.51±7.63	39.54±5.62	28.16±5.08	1.55±0.46	18.24±8.74	2.84±1.75
Lung cancer	153	66.82±8.56	33.37±7.84	27.31±7.43	1.24±0.54	15.15±7.25	5.85±1.67

Table 2. Comparison Between Patients in Different Clinical Stage of NSCLC Regarding Value of T Lymphocyte Subgroup ($\bar{\chi}\pm s$, %)

Groups	Cases	$CD3^+$(%)	$CD4^+$(%)	$CD8^+$(%)	$CD4^+/CD8^+$	NK cell	Treg cell
Control	50	73.51±7.63	39.54±5.62	28.16±5.08	1.55±0.46	18.24±8.74	2.84±1.75
Staged I~II Lung Cancer	67	71.53±5.13	37.61±7.84	26.88±6.74	1. 34±0.66	15.73±5.15	3.45±1.43
Staged III~IV Lung Cancer	86	65.42±7.72	30.59±8.63	27.89±5.89	0.98±0.48	14.15±6.67	6.25±1.08

suspension before detection. Computer test. CD3+, CD4+ cell was designated as T helper cells (Th), CD3+CD8+ cell as T suppressor cell (Tc), CD3-CD16+56+ cell as NK cells.

Detection of Treg Heparin anticoagulated peripheral blood 100 μL, with 10 μl CD4-FITC and CD25-PE antibody, and incubated under 4 °C for 30 min in a light resistant container; adding 1 ml ammonium chloride for 10 min. Centrifugation was set as 1200 RPM/min for 5 min. PBS washing for three times. After that, put 1 mL Fixation/Perm buffer for 45 min, after washing add 10μL APC labeled rat Foxp3 monoclonal antibody and incubated in a light resistant container in 4 °C for 30 min. After washed by buffer for two times and put in 500μl PBS suspension, then sumbit for test. CD4+CD25+Foxp3+ cells were recognised as Treg cells.

Statistical analysis

SPSS13.0 statistical software was used for statistical analysis. Data was expressed as $\chi\pm s$; comparison between groups was conducted using univariable analysis of variance. Statistically significant difference is significant when p value <0.05.

Results

Compared with healthy controls, CD3+, CD4+, NK cell number and CD4+/CD8+ ratio were significantly lower in those with NSCLC (p<0. 05). However, compared with control group, level of CD8+ cell in patients with NSCLC was not different (p> 0.05) (Table 1).

CD3+, CD4+, CD4+/CD8+ and NK cell count in patients with stageI ~ II lung cancer was slightly lower than those in control group, with statistical significance (p<0. 05), and this difference was significantly lower in patients with stage III ~ IV lung cancer compared with those in control group (p< 0.01).Number of CD8+ cell was not significantly different compared with control group (p> 0.05); CD3 +, CD4 +, CD4 + / CD8 + and NK cell number in patients with III ~ IV lung cancer is significantly lower those of stgage I ~ II disease (p< 0.01) (Table 2).

Number of Treg cells in patients with lung cancer is significantly higher than that in control group (p< 0.01). In different status of patients, the number of Treg cells significantly increased in patients with stageI ~ II disease compared with control group (p< 0.01), and number Treg cell in patients with stage III ~ IV is significantly higher than those with stage I ~ II (p< 0.01) (Table 2).

Discussion

It is reported from tumor immunological study that the disorder of immunological function is closely related to the occurrence and development of NSCLC, and lymphocyte subgroup could play an important role during this process (Hakansson et al., 2003; Mattes et al., 2003; Wing et al., 2003). Flow cytometry analysis is a common technology frequently used in clinical research that is able to directly analyze the percentage of lymphocyte subgroup, so as to evaluate immunological state of patient, analysing clinical condition and predicting curative effect of patients for clinicians (Karaman et al., 2013). This research adopted flow cytometry and detected T cell subgoups, including Treg in peripheral blood of 153 patients with NSCLC, to elucidate the relationship between T lymphocyte subgoups and Treg with the occurrence/ development of NSCLC to provide important basis for diagnosis and treatment.

In T lymphocyte, CD3+ subgoups represent general level of T cell, and reflect cellular immunological state of host. CD3+ T cell is divided into CD4+ helper T cell (Th), and CD8+ cytotoxic T cell (Tc). Main function is realized by secreting lymphatic factor by Th cells, increasing and boosting immunological response, and inducing other lymphatic cell to play a role of anti-tumor effect. Decreased CD3+, CD4+ T level is considered to be asssociated with a weakened immunological system. Tc cell inhibits immunological response, especially inhibiting CD4+ and B cell function, thus plays a negative role in antibody formation and cellular immune response. Increased Tc cells is considered to be in favor of tumor growth. And tumor growth is to induce Tc increase, thus there is a positive feedback model between Tc cell increase and tumor growth (Mitropoulos et al., 1995). It is suggested that CD4+/CD8+ ratio is crucial to be kept in a dynamic balance, in terms of maintaining the immunological function stable (Dou et al., 2013). the ratio decreased is reported to link with a low immunological function (Erdem et al., 2012; Nugroho et al., 2012). Our study demonstrated that patients with NSCLC had a significantly lower level of CD3+, CD4+, and CD4+/CD8+ ratio compared with healthy controls; patients with advanced staged NSCLC had a significantly lower level of CD3+, CD4+, and CD4+/CD8+ ratio compared with those with early stage. NK cell is also designated as natural killer cells. NK cell could directly destroy tumor cells, and reduced level of NK cell will lead to decline of immunological function. Our results showed that NK

cell in NSCLC patients is significantly lower than that of healthy controls, suggesting damaged immunological function in patients with NSCLC.

Treg is a group of T cell, with effect of immunosuppression and plays an important role in suppression of autoimmune disease (Manni et al., 1989). The majority of Treg is CD4+ T cell, expressing CD25 and Foxp3 (Hanaki et al., 2003). Our results revealed that level of CD4+ CD25+ Foxp3+ Treg cells was significantly higher in patients with NSCLC than that in control group, and increased with the increase of clinical stage in patients with NSCLC, suggesting that Treg cell is associated with the occurrence and development of NSCLC to some extent.

In summary, T lymphocyte subgoups and the proportion of regulatory T cells in peripheral blood detected by flow cytometry is associated with diagnosis, treatment and prognosis of NSCLC.

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