Peripheral circulating Ts (CD8+CD28-)/CD8+ is elevated in non small cell lung cancer patients

Hailong Liang1,2, Jing Zhao2, Guosheng Xing3, Huiyun Cai2, Jing Liu5, Wenqing Wei5, Xiangyang Chu1

1Department of Thoracic Surgery, General Hospital of People’s Liberation Army, Beijing 100853, China; 2Department of Thoracic Surgery, PLA Army General Hospital, Beijing 100700, China; 3Department of Pediatrics, PLA Army General Hospital, Beijing 100700, China; 4Department of General Surgery, PLA Army General Hospital, Beijing 100700, China; 5Center Lab of PLA Army General Hospital, Beijing 100700, China

Received April 27, 2016; Accepted September 20, 2016; Epub November 15, 2016; Published November 30, 2016

Abstract: Antitumor immune response is usually inhibited by immunosuppressive tumor microenvironment. CD8+CD28- T lymphocyte (Ts; or CD8+ Treg) is another regulatory T cell apart from CD4+CD25highCD127low Treg, and it has been detected infiltrating in tumor tissue of various types as well. The aim of this study is to investigate possible role of both Tregs in peripheral circulation of non small cell lung cancer (NSCLC) patients. Twenty-eight eligible NSCLC patients participated this study, and 17 healthy volunteers plus 1 patient of pulmonary bulla and 2 patients of congenital chest wall deformity were also included in this study as control group. Peripheral circulating CTL (CD8+CD28+)/CD8+, Ts or CD8+ Treg (CD8+CD28-)/CD8+, CD4+ Treg (CD4+CD25highCD127low)/CD4+ were assayed by Flow Cytometry. We observed that Ts (CD8+CD28-)/CD8+ in NSCLC group is significantly higher than that in control group (P=0.0056). CTL (CD8+CD28+)/CD8+ in NSCLC group is significantly lower than that in control group (P=0.0013). CD4+ Treg (CD4+CD25highCD127low)/CD4+ in NSCLC group is not significantly different from that in control group (P=0.8689). In conclusion, Ts (CD8+ Treg) instead of CD4+ Treg might be an immunosuppressive factor in peripheral circulation of NSCLC patients.

Keywords: Ts, CD8+ Treg, tumor immune, peripheral circulation

Introduction

Malignant tumor consists of genetically heterogeneous cells. These neoplastic cells have been observed capable of eliciting host immune response [1-3]. However, although rare cases of tumor spontaneous regression caused by host antitumor immune attack have been reported [4, 5], in most cases tumor could naturally progress in spite of elicited immune response. During recent decades, secrets of tumor evasion from immune attack is partly uncovered in that immunosuppressive microenvironment within tumor tissue is dominant so that immune effector cells are disabled or functionally altered [6]. Among various immunosuppressive factors with tumor microenvironment (TME), regulatory T cells (Treg) have been proved playing crucial role, and their recruitment and function within tumor were well studied [7]. In healthy people, Treg is responsible for maintaining host immune homeostasis, preventing excessive immune response. It has been established that Treg plays important role in autoimmune disease, organ transplantation, inflammatory diseases, etc [8, 9]. CD8+CD28- T cell is another T cell subset with similar immune regulatory function with CD4+ Treg, which is termed as T suppressor (Ts) or CD8+ regulatory T cell (CD8+ Treg). Although CD8+CD28- T cell is less focused on compared with its counterpart-CD4+ Treg, its immunosuppressive function has been emphasized during recent decade, and its role in organ transplantation [10], autoimmune diseases [11], inflammatory diseases [12] and cancer has been reported.

Evidences suggest Ts might be another important immunoregulatory cell type suppressing antitumor response. Ts cells were detected infiltrating within tumor tissue in various tumor type; purified CD8+CD28- T cell from tumor-
Peri... circulating Ts in NSCLC

infiltrating lymphocyte could inhibit T cell proliferation and cytotoxic T cell activity in vitro [13]. Currently studies on Ts cell’s role in tumor is very few. Considering tumor could exert systemic effect to facilitate its progression and metastasis, in this study, we focus on peripheral circulating immunosuppressive factors by investigating proportional changes of CD8+CD28-/CD8+ T cell in peripheral circulation of non small cell lung cancer (NSCLC) patients, aiming to evaluate its systemic influence in tumor, and provide more collaborative evidence to establish its role in tumor.

Materials and methods

This study was approved by Research Ethics Committee of both General Hospital of PLA and General Hospital of Bei Jing Military Command, and carried out in accordance with The Code of Ethics of the World Medical Association. Informed consent were obtained from all participants enrolled in this study, and individual informations were protected. From November 2015 to February 2016, eligible NSCLC patients who were admitted to either General Hospital of Bei Jing Command or General Hospital of PLA were included as NSCLC group in this study. The inclusion criteria were as follows: 1. being clinically diagnosed as lung cancer; 2. being newly diagnosed without receiving any antitumor therapy previously; 3. without acute or chronic inflammatory disease during study; 4. without suffering from immunodeficiency condition; 5. without suffering from immune-related disease; 6. without history of long-term receiving immunity influencing drug therapy. The exclusion criteria were as follows: 1. being pathologically diagnosed as benign disease or small cell lung cancer (SCLC); 2. without pathologic diagnosis; 3. receiving incomplete examinations; 4. being with other concomitant malignancy.

Besides, 20 persons were also included in this study as healthy control group. Among them, 1 person was diagnosed as pulmonary bulla, 2 persons were diagnosed as congenital chest deformity. They were all admitted in General Hospital of Bei Jing Command, while other members of control group were healthy volunteers. The inclusion criteria for control group were as follows: 1. without any type of malignant tumor; 2. without acute or chronic inflammatory disease during study; 3. without suffering from immunodeficiency condition; 4. without suffering from immune-related disease; 5. without history of long-term receiving immunity influencing drug therapy.

Peripheral blood sample

Peripheral venous blood were obtained from patients at morning and stored in heparin-coated tube, then blood samples were transferred immediately to laboratory.

Flow cytometry assay of proportion of Ts, CTL, CD4+ Treg

The reagents we used for immunostaining were as follows: FITC conjugated anti-CD4, PE-conjugated anti-CD25, ECD-conjugated anti-CD45, PC5-conjugated anti-CD127, PC7-conjugated anti-CD3, FITC-conjugated anti-CD8, PE-conjugated anti-CD28. (all reagents were commercially bought from Beckman Coulter Corporation, USA).

Cell staining

Blood samples were stained according to manufacturer’s instruction, the following panel were designed as staining combinations: 1: CD4-FITC/CD25-PE/CD45-ECD/CD127-PC5/CD3-PC7; 2: CD8-FITC/CD28-PE/CD45-ECD/CD3-PC7.

Quantification by flow cytometry

After staining, blood samples were assayed by a 5-colored uni-laser Flow Cytometer (Beckman Coulter FC500). Data analysis were processed with Kaluza software (Beckman Coulter Corporation).

The combinations of antibodies represent as follows: CD3+CD8+CD28- for T suppressor (Ts) or CD8+ Treg, CD3+CD8+CD28+ for Cytotoxic T lymphocyte (CTL), CD3+CD4+CD25highCD127-low for CD4+ regulatory T lymphocyte (CD4+ Treg).

Statistical analysis

The data are presented as mean or mean ± SD. To compare respective proportion of Ts, CD4 Treg, CTL between 2 groups, student t test was used. P values less than 0.05 is considered sig-
Peripheral circulating Ts in NSCLC

Results

General characteristics of participants

There were 28 eligible NSCLC patients participating this study. Among them, 14 cases were male, 14 cases were female; 22 cases were adenocarcinoma, 4 cases were squamous cell carcinoma, 2 cases were adenosquamous carcinoma. The average age for NSCLC group and control group are respectively 60.82 ± 12.46 VS 25.21 ± 8.17 (P=0.001) (see Table 1).

Flow cytometry assessment of Ts, CTL, CD4+ Treg: (Figure 1)

Ts or CD8+ Treg (CD8+CD28−): Proportion of Ts among CD8+ T lymphocyte in NSCLC group is significantly higher than that in control group (46.31% ± 16.11% VS 32.83% ± 14.73%, P=0.0056); (Figure 2).

CTL (CD8+CD28+): Proportion of CTL among CD8+ T lymphocyte in NSCLC group is significantly lower than that in control group (50.84% ± 16.83% VS 67.17% ± 14.73%, P=0.0013); (Figure 3).

CD4+ Treg (CD4+CD25highCD127−): Proportion of CD4+ Treg among CD4+ T lymphocyte in NSCLC group is not significantly different from that in control group (6.95% ± 2.21% VS 7.06% ± 1.19%, P=0.8689); (Figure 4).

Discussion

Malignant tumor remains one of the biggest threats to human health and enormous economic burden to societies. According to latest published cancer statistics, cancer-related death worldwide in 2012 amount to 8.2 million, and newly diagnosed cases amount to 14.1 million [14]. Molecular and biologic studies reveal that neoplastic cells are genetically unstable and heterogeneous, which theoretically become target of host immune attack. Many evidences demonstrate the existence of host immune response targeting malignant tumor cells in human and animal models [1-3]. However, in most cases tumor grows wildly in host in spite of detected existence of antitumor immune response. Moreover, various types of immunotherapies aiming to augment immune function failed in most patients. During recent decade, concept of tumor microenvironment (TME) was proposed to explain the failure of antitumor immune attack against tumor cells. According to cancer immunoediting theory [15], majority of neoplastic cells were eliminated by antitumor immune response, while a genetically heterogeneous subset survived and could exploit various means to form suppressive immune milieu at tumor site so as to escape immune attack. One important pathway is to recruit regulatory T cells (Treg) from peripheral circulation (natural Treg, nTreg) and incuce topical naive CD4+ T cell converting into Treg (induced Treg, iTreg).

Treg’s role in tumor microenvironment has been well studied, and some newly developed monoclonal agents targeting to block some of its inhibitory effect achieved inspiring clinical benefit [16, 17]. In contrast to Treg, CD8+CD28− T cell termed as T suppressor (Ts) or CD8+ Treg is far less emphasized. During recent decade, Ts or CD8+ Treg, was proved capable of exerting similar immunoregulatory function with Treg. Ciubotariu etc provided direct evidence that CD8+CD28− T cell could suppress human CD4+ Th cell activation by interfering with antigen presenting cell providing necessary co-stimulating signal when bearing antigen [18]. Later,
Peripheral circulating Ts in NSCLC

Chang etc found that CD8+CD28- Ts could induce upregulation of inhibitory receptor ILT3 and ILT4 in monocyte and dendritic cell, thus reduce their expression of co-stimulatory molecules, finally cause corresponding antigen-specific Th cell into unresponsiveness [19]. Manavalan etc reported Ts could also work on endothelial cell in a similar way [20]. Recently Liu etc reported that human mesenchymal stromal cells could enhance CD8+ Treg’s immunoregulatory function. More importantly, they proposed that CD8+ Treg’s function included inhibiting naive CD4+ T cell proliferation and activation, reducing production of IFN-γ by activated CD4+ T cell and inducing apoptosis of activated

Figure 1. Flow Cytometry distribution of Ts, CTL, CD4 Treg (A, B). (A) Coexpression of CD8 and CD28, Ts is indicated as CD8+CD28-, CTL is indicated as CD8+CD28+; (B) Coexpression of CD25 and CD127, CD4+ Treg is indicated as CD25highCD127low.

Figure 2. Comparison of Ts. In NSCLC group, peripheral circulating Ts (CD8+CD28-) is significantly higher than those in control group (46.305 ± 16.116 VS 32.833 ± 14.732, P=0.0056).

Figure 3. Comparison of CTL. In NSCLC group, peripheral circulating CTL (CD8+CD28+) is significantly lower than those in control group (50.837 ± 16.834 VS 67.172 ± 14.729, P=0.0013).

Figure 4. Comparison of CD4+ Treg peripheral circulating CD4+ Treg (CD4+CD25highCD127low)/CD4+ is not significantly different between NSCLC group and control group (6.954 ± 2.208 VS 7.058 ± 1.194, P=0.8689).
Peripheral circulating Ts in NSCLC

CD4+ T cell. They also found that CD8+ Treg’s working manner might be IL-10 and FasL dependent [21].

Presently Ts or CD8+ Treg has been proved to play a role in various diseases including organ transplantation, autoimmune disease, inflammatory disease and cancer. However, relevant studies are just beginning, especially for studies on CD8+ Treg’s role in tumor which are few. Pages etc reported that CD8+CD28- T cells infiltrated in human colorectal cancer tissue, nevertheless, these cells seemed to be cytotoxic rather than immunosuppressive, and correlated with better prognosis [22]. Filaci etc made comprehensive study on tumor-infiltrating CD8+CD28- T cell function. They found that tumor-infiltrating CD8+CD28- T cell were observed in various tumor types, and purified tumor-infiltrating CD8+CD28- T cell exhibited suppressive function on T cell proliferation and cytotoxicity [13]. Parodi etc recently reported that both CD4+ Treg and CD8+ Treg were found infiltrating in human bladder and renal cancer tissues, while constitution of both Tregs within tumor mainly by CD4+ Treg or CD8+ Treg varied in different cases. They also reported that more Treg than effector T cell within tumor is correlated with tumor relapse [23]. All these findings collectively establish that Ts or CD8+ Treg cell play immunosuppressive role in tumor microenvironment.

In this study, we detected that CD8+CD28-/CD8+ T cell in peripheral circulation of NSCLC patients was significantly higher than that of healthy people, while CD4+ Treg/CD4+ T cell in peripheral circulation did not differ between NSCLC patients and healthy people. This finding indicates that Ts or CD8+ Treg instead of CD4+ Treg is an immunosuppressive factor in peripheral circulation, which has two-fold meanings: firstly, since local immune status is closely associated with host peripheral circulation immune status, such as immune cells recirculation or recruitment, theoretically effector T cells infiltrating tumor tissue could be impaired or even inactivated before their arriving at tumor by Ts or CD8+ Treg. If it were proved, then elevated Ts in peripheral circulation would undoubtedly be an important contributing factor for tumor immunosuppressive milieu and also potential target for immunotherapy. Presently relevant study is lacking, further investigations on peripheral circulation effector T cell’s function in tumor patient would provide direct evidences; secondly, considering hematogenous metastatic tumor cells transport through blood vessels to arrive at distant organ, Ts cell might facilitate metastasis by impairing circulating CTL or Th cell function so that tumor cells would have more chances safely arriving in stead of being captured and killed. of course it also need experimental evidences to support. If true, it would be new target for clinical prevention and control of metastasis.

There are two possible flaws in this study. The first is that when setting control group, we did not take age-matching into consideration. According to Simone and colleagues’ study, frequency of CD8+CD28- T cell in peripheral circulation increases with aging [24]. Till now, no other studies report similar result. If it is true, the influence of aging should be excluded as a disturbing factor. As it is impossible to discriminate influence of aging in this study, if necessary, setting an age-matched control group and making comparison again could solve this problem. The second flaw is that some studies proposed that tumor-infiltrating CD8+CD28- T cells are functionally heterogeneous, being cytotoxic or immunoregulatory, moreover, there is no known distinguishing marker to discriminate them [25, 26]. So a question rises naturally: is increased CD8+CD28- T cells in peripheral circulation of NSCLC patients mainly cytotoxic or immunoregulatory? Further studies assaying circulating CD8+CD28- T cell’s function could ascertain its character.

In conclusion, we observed that CD8+CD28-/CD8+ T cell in peripheral circulation of NSCLC patients is significantly elevated compared with healthy people, which indicates that Ts or CD8+ Treg might be peripheral immunosuppressive factor.

Acknowledgements

This study was supported by Lab of Department of Pediatrics and Lab of Department of General Surgery, General Hospital of Bei Jing Military Command.

Disclosure of conflict of interest

None.
Peripheral circulating Ts in NSCLC

Address correspondence to: Xiangyang Chu, Department of Thoracic Surgery, General Hospital of People’s Liberation Army, Hai Dian District, Fu Xing Road 28#, Beijing 100853, China. Tel: 136-61248731; Fax: 0086-010-66938312; E-mail: drchu0521@163.com

References

Peripheral circulating Ts in NSCLC


