SciMedCentral

Journal of Immunology & Clinical Research

Perspective

Peripheral Blood Derived Treg Cells could Serve as Prognostic Marker of the Metastatic Cancer where TIL Derived Tregs are Not Accessible/ Available for Analysis

Nitya G. Chakraborty*

Department of Medicine, University of Connecticut, USA

***Corresponding author**
Nitya G. Chakraborty, Department of Medicine, University of Connecticut, Farmington, CT 06030-3229, USA, Tel: 860-679-1446; Email: chakraborty@uchc.edu

Submitted: 22 August 2017

Accepted: 11 September 2017

Published: 13 September 2017

ISSN: 2333-6714

Keywords
- Treg Cells
- Metastatic Cancer
- Natural Killer cells
- Natural Killer T Cell

Abstract

Although generating a robust and long-lived cytotoxic T lymphocyte (CTL) response to tumor associated antigens faces many constraints, adoptive immunotherapy has impressive clinical responses. The negative role of regulatory T cells (Treg) on immune responses, especially against "self" antigens (tumor associated antigens), has turned out to be a major constraint. In a recent report with tumor infiltrating lymphocyte (TIL) derived Treg cells fromhuman breast cancer and colon cancer, De simone's group has clearly demostrated a poor prognosis of patients, associated wth increased number of functional Tregs from TIL. In this report we discuss, in human prostate cancer, that peripherally induced Treg (pTreg) cellsplay a major role in the down regulation of effective immunity against cancer whether those effectors are from innate immune system (NKT or NK cells) or from adaptive immunity via antigen specific cytotoxic T lymphocytes (CTL). Increase of peripheral blood (PBL) derived Treg cell number is directly related to higher gleason scores. PBL derived T cells, natural killer T cells (NKT) and natural killer cell (NK) showed significantly poor activity. Treg cells isolated from the patients with higher gleason score also showed increased suppressive activity in proliferation of conv CD4+ T cells.

INTRODUCTION

Recently De Simone et al. [1], demonstrated that tumor-infiltrating Treg cells display specific gene signatures that were also validated at the single-cell level. They have shown that tumor-infiltrating Treg cells were highly suppressive, up-regulated several immune-checkpoints, and expressed on the cell surfaces specific signature molecules. Their findings provided insights into the phenotypic, molecular and functional characteristics of human tumor-infiltrating Treg cells and define potential targets to improve tumor immunotherapy.

Metastatic diseases remain a serious challenge in cancer therapy. Regulatory T lymphocytes (Treg) appear to be a significant inducer of tolerance [2-7]. Here we discuss in human prostate cancer, that Treg cells play a major role in down regulation of effective immunity against cancers whether those effectors are from innate immune system (NKT or NK cells) or from adaptive immunity via antigen specific cytotoxic T lymphocytes (CTL) [2-9]. Lack of an effective way to engage CD4+ T cells as helper cells as well as effector cells in mitigating T reg cell activities has turned out to be the major problem. Dr. Gershon's group was the first to describe T suppressor cells in cancer [2]. Then Dr. North's group demonstrated that CD4+ T cells could function as suppressor cells in an animal tumor model [3]. We extended that basic observations and proved in clonal level that CD4+ T cells could function as suppressor cells in the human melanoma model [4,5]. These observations led to a substantial amount of work by others [6-9]. Dr. Sakaguchi's group revived the topic of suppression by showing that a set of T cells emerging from the thymus naturally as CD4+ CD25+ T cells (hence called natural Treg (nTreg now called tTreg) has a critical role in the control of autoimmune pathology [6]. It is now clear that regulatory T cells are also generated in the periphery and are now referred to as p Treg cells [10].

TREG CELL NUMBER AND PROGNOSIS OF THE DISEASE

Since Treg (CD4+ CD25+ Foxp3) cells play important role in down regulating tumor specific CTL response, we compared the Gleason score (prognostic marker) of prostate cancer patients' disease with the number of Treg cells present in PBL of the respective patient. We found that the patients showing higher Gleason score also showed higher number of Treg cells in PBL as shown in Figure 1. When compared with of CD8+ T cell reactivity

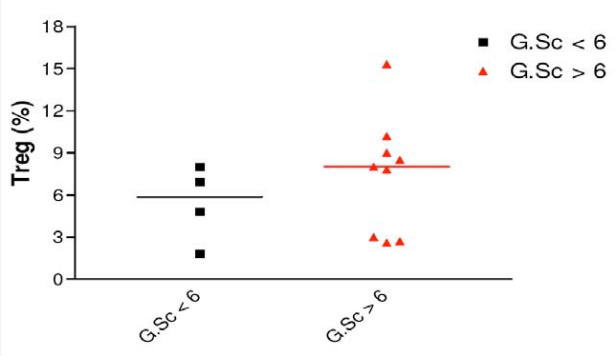

Figure 1 Comparison of Gleason score and percent of T reg cells from the Prostate cancer patients. The patient having lowest number of T reg cells as in the Figure 1, is a white American surviving with minor or no complications for 10+ years. Where as the patient showing highest number of circulating T reg cells was an African American, survived with serious complications for 18 months after diagnosis. This data along with the results from different laboratories suggest that Treg might play a role in non-reactivity of CD8+ CTL (or non reactivity of helper type-1 CD4+ cells) followed by progression of the disease.

against prostate antigens, we observed that the patients having Gleason score from 5 or less had existence of antigen reactive CD8+ cells (CTL) and the reactivity of those cells was detectable for a prolonged period (checked two to three times in a period of two years). Whereas the CD8+ cells taken from the patients who had Gleason score 6 to 10, did show some initial reactivity against PSA peptides but that reactivity was lost as the number of functional T reg cells increased in PBL over the time of 2 years [11].

STUDY WITH NATURAL KILLER (NK) AND NATURAL KILLER T CELL (NKT)

In innate immune system NK cells and NKT cells play very important role in anti tumor response. One interesting observation by Carnaud C, et al. (Cutting edge article in J Immunol) [12], where they showed that NKT cells rapidly activate NK cells in mouse and in human system. With our limited scope we wanted to see whether NKT cells could activate autologous NK cells in their effector phase also. We isolated NK cells by positively selecting CD56+ cells by using beads from Dynal (OSLO, Norway), from patients' and normal donors' PBL. We obtained an approximately 90% pure population of NK cells. We then separated the CD3+ CD56+ cells by positive selection with CD3+ dynal beads. We tested these two types of cells in NK killing assay using natural killer cell sensitive targets. Table 1 shows that the NKT cells do not show any significant killing of NK targets while CD56+ and CD3- NK cells showed significant killing. In a separate cytotoxicity assay we did mix NKT(CD56+ CD3+ cells) with (CD56+ CD3-) NK cells at the effector phase. The percent cytolytic activity of CD56+ NK cells was found to be increased significantly by the addition of double positive cells (Table 2) even when as low as ratio of 100 NK: 1 NKT cells was used. It appears that NKT cells might also enhance the activity of NK cells even at the effector phase. When the NK cells were taken out from a patient with higher Gleason score and higher number of T reg cells in PBL we detected significantly lower NK cell activity and no help from the NKT cells separated from the same patient (Table 1,2). The patient we tested here had a very high G-score and also very high Treg cell number. This observation with additional patients and normal donors and further comparing that with total number of T reg cells in PBL should be helpful to determine the prognosis and possible immunotherapeutic intervention for metastaic solid tumors.

STUDIES WITH CONV CD4+ CD25- CELLS AND T REG (CD4+ CD25+) CELLS

Figure 2 shows a standard Treg assay in a mix culture of T regs and CD4+ CD25- T cells (Tconv) as responder cells upon stimulation with anti CD3 antibody with or without autologous dendritic cells.

T and B lymphocytes could specifically recognize tumor cells [13]. T lymphocytes recognizing tumor associated antigen-derived peptides presented by major histocompatibility complex

Table 1: Comparison of killing of NK sensitive target by NK cells and NKT cells.

Effector cells from	% Lysis at E:T			
	By NK cells		By NKT cells	
	5:1	2.5:1	5:1	2.5:1
Normal Donor 1	20	16	2	0
Normal Donor 2	24	21	3	1
Patient	4	0	0	0

Table 2: Increase of NK killing activity with donor 1 and pa. NK cells when mixed with NKT cells.

Effector cells	Target lysis at E:T =2.5:1
Donor 1 NK	16
Donor 1 NK +NKT (100:1)	25
Donor 1 NK + NKT (10:1)	36
Patient NK	0
Patient NK + NKT (10:1)	0

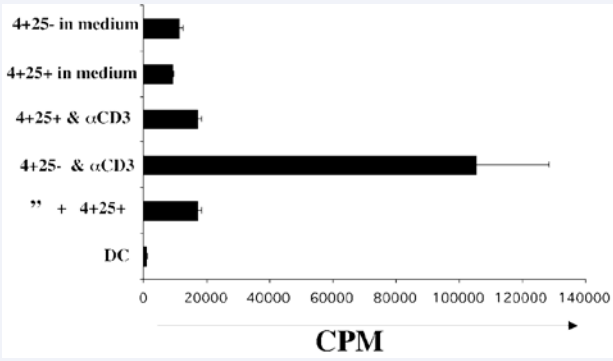

Figure 2 Standard Treg cell assay.
In vitro proliferation assay with CD4+CD25- T cells as responder cells. CD4+ CD25+: CD4+ CD25- = 1:10. As shown, the CD4+ CD25+ cells were anergic to stimulation but they suppressed the proliferation of the CD4+ CD25- T cells. Suppressive activity of CD4+ CD25+ cells further increased in the presence of DC, the last line in the Figure.

(MHC) molecules play a central rolein immunotherapy [14]. In fact, anti-tumor CD8+ T cell responsesmight arise automatically in cancer patients but those are disabled by varying conditions in the tumor microenvironment and tumor progresses [15]. The immunosuppressive mechanisms depend on the integrated action of in filtrating leukocytes, lymphocytes and the tumor that upregulate a range of modulatory molecules,collectively called immune checkpoints [16]. Therefore, thesearch for antagonistsof inhibitory molecules isprimary goal of current anti-tumor research [17]. In several clinical trials with CAR T cells being tested with reasonable success to overcome all the above mentioned constraints [18]. Treg cells are found to express very high level of these inhibitory molecules and thenumber of these Treg cells increases also in circulation as the disease progresses. In this regard we argue that tumor tissue might not be accessible by the surgeons to get TIL derived Tregs but PBL derived Tregs could serve as prognostic marker and could be usefull for doing some simple experiments for identifying target molecules for the improvement of therapeutic outcome.

REFERENCES

1. De Simone, Alberto A, Grazisa R, Paola G, Valeria R, Claudia P, et al. Transcriptional Landscape of Human Tissue Lymphocytes Unveils Uniqueness of Tumor-Infiltrating T Regulatory Cells. Immunity. 2016; 45: 1135-1147.
2. Gershon RK, Kondo K. Infectious immunological tolerance. Immunology. 1971; 21: 903-914.
3. Mills D, North RJ. Expression of passively transferred immunity against an established tumor depends on generation of cytolytic T cells in recipient. Inhibition by suppressor T cells. J Exp Med. 1983; 157: 1448-1460.
4. Mukherji, Chakraborty NG, Sivanandham M, Nashed AL, Sporn JR, Ergin MT. Clonal analysis of cytotoxic and regulatory T cell responses against human melanoma. J Exp Med. 1989; 169: 1961-1976.
5. Chakraborty NG, Twardzik DR, Sivanandham M, Ergin MT, Hellstrom KE, Mukherji B. et al, Autologous melanoma-induced activation of regulatory T cells that suppress cytotoxic response. J Immunol. 1990; 145: 2359-2364.
6. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995; 155: 1151-1164.
7. Beyer M, Schultze JL. Regulatory T cells in cancer. Blood. 2006; 108: 804-811.
8. Maloy KJ, Powrie F. Regulatory T cells in the control of immune pathology. Nat Immunol. 2001; 2: 816-822.
9. Piccirillo CA, Thornton AM. Cornerstone of peripheral tolerance: naturally occurring CD4+CD25+ regulatory T cells. Trends Immunol. 2004; 25: 374-380.
10. Karimi S, Chattopadhyay S, Chakraborty NG. Manipulation of regulatory T cells and antigen-specific cytotoxic T lymphocyte-based tumour immunotherapy. Immunology. 2015; 144: 186-196.
11. Chakraborty NG, Robert LS, Shikhar M, Elizabeth L, Pamela T, Jonathan RS, et al. Recognition of PSA-derived peptide antigens by T cells from prostate cancer patients without any prior stimulation. Cancer Immunol Immunother. 2003; 52: 497-505.
12. Carnaud C, Lee D, Donnars O, Park SH, Beavis A, Koezuka Y, et al. Cutting edge: Cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. J Immunol. 1999; 163: 4647-4650.
13. Mariam JH, Eirini T, Karl SP, Sergio AQ, Charles S. Curr Opin Pharmacol. 2013; 13: 497-503.
14. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012; 12: 252-264.
15. Galluzzi L, Aitziber B, Oliver K, Laurence Z, Guido K. Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. Cancer Cell. 2015; 28: 690-714.
16. Munn DH, Bronte V. Immune suppressive mechanisms in the tumor microenvironment. Curr Opin Immunol. 2016; 39: 1-6.
17. Zitvogel L, Galluzzi L, Smyth MJ, Kroemer G. Mechanism of action of conventional and targeted anticancer therapies: reinstating immunosurveillance. Immunity. 2013; 39: 74-88.
18. Jackson HJ, Rafiq S, Brentjens RJ. Driving CAR T-cells forward. Nat Rev Clin Oncol. 2016; 13: 370-383.

Cite this article

Chakraborty NG (2017) Peripheral Blood Derived Treg Cells could Serve as Prognostic Marker of the Metastatic Cancer where TIL Derived Tregs are Not Accessible/ Available for Analysis. J Immunol Clin Res 4(2): 1044.