Review Article

A Systemic Review of T Regulatory Cells and Their Role In Hodgkin and Non-Hodgkin Lymphoma

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Abstract

Regulatory T cells (Tregs) know for monitoring and regulating the immune response in healthy individuals. In hematological malignancies, Tregs cells exert an immunosuppressive effect thus playing an important role in tumor progression and spread. It is a systemic review focusing on evolution and prognostic role of Tregs in hematological malignancies and the results are very conflicting. Different tissues (peripheral blood, Lymphnode tissue and bone marrow) were used for studies with applications of different methods like Flow cytometry on whole blood / isolated peripheral blood mononuclear cells with different gating techniques, Immunohistochemistry using different panel of monoclonal antibodies, which partially explained the confrontation of results. It is of particular importance to mention above finding to solidify the requirement of use of standardized approaches in study of Tregs in hematological malignancies and in cancer.

Keywords:

T regulatory cells, Hodgkin Lymphoma, Non Hodgkin Lymphoma, Cytotoxic T lymphocyte Antigen-4

Introduction

T regulatory (Treg) cells are a subpopulation of T cells defined as CD4+ CD25+cells which have immunosuppressive activity that modulates the immune system by various mechanisms. Physiological role of Treg is to maintain the tolerance to self antigen to prevent development of autoimmune diseases. Treg comprises about 1-4 % of circulating CD4+ in peripheral blood.1

With widely accepted theory of immune surveillance, the role of Treg in cancer immunity has been redefined.2 There are two school of thoughts as far as the role of Treg is concerned in tumor immunity with most of studies reported that increased Treg frequency in malignancy is associated with poor outcome3- 10, however many other studies also suggest that increased Treg

frequency at the site of tumor is associated with better prognosis.11-13 As far as the hematological malignancy considered, the role of Treg is debatable. Conflicting results are obtained in B-NHL and HL. Some studies demonstrate Treg association with poor prognosis with reduced overall survival while other suggest a better prognosis.14-19 In this article we will review the role of Treg in hematological malignancies in malignant lymph node and in peripheral blood.

Discussion

1. T-regulatory cells- evolution through decades:

The immune-suppressive character of T-lymphocytes has been known for decades. It was in 1970, when Gershon and Kondo20 described for the first-time so-called suppressor TD cell (Thymus derived), when they transferred antigen-specific tolerance to thymectized mice by injecting T-cell exposed to certain antigen. They also found that this tolerance can be circulated to surrounding T-cells and B-cells. In the following years many studies were carried out to characterize these cells but in the absence of any specific marker and conflicting result concept of Treg become of lesser interest. In 1995 Sakaguchi et al identify a population of CD4+ T cells which highly express CD25 and help in preventing auto immune disease in mouse model which is now termed as Treg25. Many studies afterward highlighted the diverse nature of Treg and their role in development of autoimmunity and in cancer progression or suppression.

2. Important Milestone in Tregs evolution

Identification of so called TD(Thymus derived) cells with immunosuppressive activity	*1970, R. K. Gershon and k. Kondo* [20]
Discovery of CD4+ subpopulation with high CD25 expression and a role in prevention of autoimmune diseases-These were termed as Tregs	*1995, Sakaguchi at el*[21]
Identification of direct suppressive function of Tregs by cell-to-cell contact	*1998, Takahashi, Kuniyasu*[22] *1998, Thornton and Shevach*[23]
Discovery of Foxp3 gene in Scurfy mice which developed autoimmune disease	*2000, Brunkow &Jeffery at el*[24]
Discovery that Foxp3 is a key molecule essential for TR cell development and function	*2003,* Khattri *and Tom Cox*[25]. *2003, Fontenot and Gavin.*[26] *2003* *Hori, Nomura and Sakaguchi*[27]

3. Current knowledge

Function

The main function of Treg is to regulate the unwanted immune response. This can be achieved by either directly by cell to cell contact inhibition or by release of inflammatory cytokines. Direct mechanism includes cell- cell contact between Treg and target cell and delivery of suppressive factors through gap junctions like cyclic AMP. Cytolysis of target cells is also included in this mechanism which is achieved by the help of Membrane bound TFG-B 28-34. Another mechanism includes modulating the antigen presenting cells by direct cell-cell to contact via engagement of cytotoxic T lymphocyte antigen-4 (CTLA-4) on Tregs with B-7 ligand on APC. CTLA-4 is an inhibitory relative of the T cell costimulatory molecule CD28. While CD28 pathway induces T cell

activation, CTLA-4 provides an immunoregulatory function, suppressing the T cell response. Despite their opposing functions, both CD28 and CTLA-4 interact with the same shared ligands CD80 (B7.1) and CD86 (B7.2). The superior affinity of CTLA-4 for both ligands is balanced by its predominantly intracellular location contrasting with CD28 which is constitutively expressed at the cell surface. A diverse array of mechanisms has been proposed to account for the inhibitory function of CTLA-4; these include competing with CD28 for binding to their shared ligands, downregulating ligand expression and transmitting inhibitory signals.

The indirect mechanism is by secretion of soluble factors or cytokines namely IL-10, TGF-B and IL-35. Interleukin 10 causes the long lasting anergy state of effector T-cell and down regulates the co stimulatory adhesion molecule and MHC-II on APC. While TFG-B Blocks differentiation of naive T cells to effector T –cells by inhibiting IL-2. IL -35 suppresses T cell proliferation and further stimulates Treg to produce the IL-10.35-36

It is proposed that in cancer Treg show an increased tendency to infiltrate and accumulate at the site of tumor. This increased tendency of accumulation is attributed to the various chemokines. The chemokine receptors CCR4 and CCR8 are shown to be expressed by Tregs and the CCR4 ligand CCL22 has been shown to be produced by both tumor cells and tumor-infiltrating macrophages. Expansion of Tregs can also be occurred by the help of IL-2 secreted by effector T cell. As IL-2 is essential for development and homeostasis of Tregs.37-39 Two major subsets of Tregs have been described. Natural Tregs arise from thymus and after many changes in the thymus they are released in to the peripheral circulation. However, the precise mechanism for their development in the thymus and emigration to the periphery are still unclear. Various evidences indicate that naturally occurring Treg cells arise in the thymus upon interaction with medullary DCs during the process of negative selection and populate the periphery. Another subset of Tregs called Inducible or Adaptable Treg generated from naive T cells by antigenic stimulation under the influence of various chemokine factors like IL-10, IL-2 and TGF-B.

Induced regulatory T cells arise during inflammatory processes in the context of infectious or malignant diseases and might be highly relevant in patients with hematological malignancy40. While the natural Tregs expresses the stable form of FOXP-3, inducible Treg only express FOXP-3 after conversion. Induced regulatory T cells differ from thymus-derived Tregs in their dependency on cytokines but not in their suppressive capacity or proliferative response upon antigenic stimulation.41-42 Inducible Tregs are further subdivided into- CD4+Tr1, Th3 Tregs, CD8+Treg and Treg follicular.

The micro environment is what responsible for the phenotypic characteristic of Tregs.43-44 It has been found that Tregs is not the terminal point in the line of differentiation and they can further be transformed in the T-effector cells depending on the microenvironment which allow them to differentiate in the different direction and do multitasking,45-46 however it also make difficult to recognize precisely these cells in the intermediate stage leading to their complex role in hematological malignancies

4. Immunophenotype

After Sakaguchi described CD4+ cells with immunosuppressive activity, Bach-Allan demonstrate that these suppressive CD4+ cells express high levels of CD 25 using cell culture, cytokine analysis and Flowcytometery evaluation. Most Treg cells express CD25 (70–80%) 47 but the existence of CD25-negative Treg cells has been reported by many author and CD25 expression is not exclusive to Tregs cells and also expressed by effector T cells recently exposed to foreign antigen.48-49 This lack of specificity has overcome by identification of more specific marker FOXP-3, uniquely expressed by Tregs in mouse. The transcription factor Forkhead box P3 (FoxP3) is considered the most specific marker for Treg cells. FoxP3 acts both as a repressor and an activator of gene transcription and binds over 700 genes. It is known to repress the gene expression of IL-2, CD127 (IL7R), tumor necrosis

factor-α and interferon-γ, and to enhance the expression of CD25 and CTLA-4.Though in human FOXP-3 also expressed by conventional T-cells without suppressive features activated by antigenic stimulation.50-53 Another marker CD127 is a surface marker and expressed in very low quantity by Treg is considered by many as a genuine Treg marker. Its expression is down regulates in Treg by transcriptional factor Foxp-3. Given the fact that CD127 is a cell surface marker it can be used to distinguish the CD25high CD127low Treg cells from the CD25high CD127high activated T cells.54Additional markers expressed by Treg cells are cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), 55-56glucocorticoid-induced tumor necrosis factor receptor2957 and lymphocyte-activation gene 3. These markers are constitutively or highly expressed on Treg cells but suffer the same fate as CD25 by being up regulated on conventional T cells upon activation.54-55

5. Role of Treg in hematological malignancy

As the knowledge about Tregs evolves, more studies focused the role of Treg in cancer. Concept of Treg in hematological malignancies developed in recent years.

Treg in B-NHL

As far as B cell NHL considered the role of Tregs in disease progression and prognosis is contradictory. (Table.1 and Table.2)

Table 1: Treg In B-NHL

Study	Disease	Sample size	Marker	Technique	Treg status	Functional role	Interpretation
Vassilikin et al (2017)[67]	CLL	44	CD4, CD25, Foxp3, CD127	FCM on peripheral blood	Increased	Suppression of T effector cells & Proliferation of CLL clone	Increased frequency associated with Advanced disease (Binet)
Wei wu et al (2015)[16]	B-NHL	30	CD4, CD25, IL-10/TGF-B	FCM &ELISA on peripheral blood and serum	Increased	Non	Increased frequency associated with disease progression and after CR show increasing percentage of Treg than normal control
Chang et al (2015)[15]	DLBCL	77	CD4, CD25	FCM on peripheral blood	Increased	Non	Increased frequency associated with poor prognosis and higher IPI score
El-dien et al (2016)[68]	DLBCL	70	Foxp3	IHC on LN Bx	Increased	Increased intra-tumoral Tregs have anti tumor effect	Increased frequency associated with better prognosis
Ahmad Barak & Hotem (2011)[69]	B-NHL	45	CD4, CD25, Foxp3	FCM on peripheral blood	Increased	Non	Increased frequency associated with Advanced disease and marrow infiltration
Christopolus et al (2011)[70]	Indolent B-cell Lymphoma	33	CD4, CD25	FCM peripheral blood	Decrease overall Tregs in Peripheral blood, however a relative increased is seen due to decreased naive T-cell population	T effector cell dysfunction	Decreased frequency associated with better prognosis

Treg- T regulatory cell, CLL-Chronic lymphocytic leukemia, DLBCL-Diffuse large B cell lymphoma, B-NHL – B- Non Hodgkin lymphoma, FCM-Flowcytometery, IHC-Immunohistochemistry, LN Bx- Lymph node biopsy, CR- Complete remission, IPI score- International prognostic index score

Chronic Lymphocytic leukemia is associated with marked deregulation of immune system. Early studies of T cell abnormalities in and immune deregulation were published in 1970-80, however could not pin point the Tregs.58-59 Later studies established the correlation of increased frequency of Tregs with aggressive clinical features and adverse prognosis.

Table 2: Treg In NHL

Study	Disease	Sample size & type	Marker	Technique	Treg status	Functional role	Interpretation
T zankov et al(2007)[14]	B-NHL and HL	739 cases of B-NHL and 280 of HL	Foxp-3	IHC on LN Bx	Increased	Non	**In FL ,GC-DLBCL & HL-** Increased Tregs associated with better prognosis and disease and failure free survival **Non GC-DLBCL**- Negative prognostic effect
Dasgupta et al(2014)[60]	CLL	130	CD4, CD25, Foxp3, CD127	FCM peripheral blood	Increased	Non	Correlation with ZAP-70 and CD-38 expression and poor prognosis
D'Arena et al (2011)[18]	MBL &CLL	56 patients of MBL and 74 patients of CLL	CD4, CD25, CD 127	FCM peripheral blood	Increased	T effector cell dysfunction	Correlated with Rai 0 stage at higher risk-requiring therapy
Biancotto et al(2012)[71]	CLL	21	CD4, CD25, Foxp3	FCM on peripheral blood	Increased	Slightly reduced T effector functional activity	Correlation with ZAP-70 and CD-38 expression and poor prognosis
Rissiki et a(2014)[72]	MBL &CLL	20 MBL cases, 20 early CLL cases (Rai 0), 20 more advanced CLL cases (Rai I-IV)	CD4, CD25, CD39, CD127	FCM on peripheral blood	Increased in highly suppressive CD39 + cells	Increased immune-suppressive effect at MBL stage with sequentially increased above effect as disease progresses from MBL to CLL	Increased Treg no. associated with poor prognosis
Carreras at el(2006)[62]	FL	97	Foxp-3	IHC on LN Bx	Increased	Non	Increased Treg are associated with improved prognosis and improved overall survival, while relapse cases show significantly low Treg numbers
Sajjan Mittal at el(2008)[73]	NHL	30	CD4, CD25, Foxp3, CD127	FCM on peripheral blood	Increased	.Defective Effector cell function .Tumour cell induce and expand CD25+ Tregs from CD25- T cells	In Increased Treg are associated with poor clinical features and high levels of LDH
Gunduz atel(2016)[66]	NHL	40	CD4, CD25, Foxp3	FCM on peripheral blood	Increased	Non	Increased Treg are associated with poor prognosis

Treg- T regulatory cell, CLL-Chronic lymphocytic leukemia, B-NHL-B-Non Hodgkin lymphoma, DLBCL-Diffuse large B cell lymphoma, HL- Hodgkin lymphoma, FL- Follicular lymphoma, GC-DLBCL- Germinal centre Diffuse large B cell lymphoma, Non GC-DLBCL-Non Germinal centre Diffuse large B cell lymphoma FCM- Flowcytometery, IHC-Immunohistochemistry, LN Bx- Lymph node biopsy, CR- Complete remission, IPI score- International prognostic index score, MBL-Monoclonal B lymphocytosis

Dasgupta et al established an optimal threshold of Treg in patient of CLL as prognostic indicator, using receiver operating characteristic (ROC) analysis and a cut off of 35cell/ul absolute no of Tregs and 5.7% Treg respectively determined. A median Treg cells percentage of 15.5% used to separate low and high risk patients.60

Giovanni Et al using the same approach in Rai 0 stage CLL patient demonstrate that the absolute no. of Tregs is an independent predictor of the time to first treatment with a cut off being 41cells/ul. These data show that the absolute no of Tregs is able to identify Rai stage 0 patients at higher risk of requiring therapy. The no of Tregs are found to be lower in MBL (monoclonal B – cell lymphocytosis) than CLL but it is higher than in normal healthy control. As the disease evolve from MBL to early stage of CLL The no of Tregs increased in sequential manner which show that suppressive Treg profile develop early during MBL and as the disease progress to overt CLL the no of Tregs increased with compromise in effector t cells functions and contribute to the

disease progression. 61

As far as other B cell lymphoma are considered, increased trafficking of Treg found in the involved lymph node along with other cells like dendritic cells and macrophages so called Tumour microenvironment. Tzankov et al showed that increased frequency of Tregs in involved lymph node is correlated with disease specific and failure free survival in follicular lymphoma as well as in germinal centre –Diffuse Large B Cell Lymphoma (DLBCL), however same reported that increased frequency is associated with negative prognostic effect in non germinal centre –DLBCL.14

Carrears et al demonstrate a conflicting result as compared to above study in follicular lymphoma and stated that increased frequency of Tregs is associated with decreased overall survival and decreased frequency is associated with refractory disease. In gastric Maltoma Tregs increased frequency is associated with better response to antibacterial eradication therapy and better response.62

Change et al studied the Tregs in peripheral blood of DLBCL patient and found that these cells are increased in no as compared to healthy control and associated with poor overall survival with high IPIS score.63

Treg in Hodgkin Lymphoma

Hodgkin lymphoma is characterized by few tumor surrounded by a profound inflammatory component, which creates a profound inflammatory tumor microenvironment. It has been demonstrated that microenvironment in Hl is dominated by T lymphocytes and these further comprises a predominance of Treg and Th-2 cells. Studies targeting the role of Tregs in HL are not many and done predominantly in affected lymph node. (Table.3)

Table 3: Treg in Hodgkin lymphoma

Study	Sample size	Marker	Technique	Treg status	Functional role	Effect on prognosis
Alvaro et al (2005)[14]	257	Foxp-3,	IHC on LN Bx	Decreased in Relapse cases	Non	Decreased Treg is associated with negative event free and disease-free survival. On follow up Increase Tregs showed improved overall survival and relapse are associated with reduced Tregs
Gunduz et al (2016)[66]	21	CD4, CD25, Foxp3	FCM on peripheral blood	increased	Non	Increased Treg in HL associated with poor prognosis and increased B-symptoms
Schrek et al (2009)[17]	87 (Relapsed/refractory cases)	Foxp-3	IHC on LN Bx	increased	Non	Higher Treg/Th2 ratio is correlated with shortened event free survival otherwise increased DFS/EFS
Korishi et al (2012)[74]	63	Foxp-3	IHC n tissue micro-array	Decreased	Non	decreased no in refractory disease is associated with poor overall survival
Treg- T regulatory cell, Th2- T helper cell-2 , HL- Hodgkin lymphoma ,FCM- Flowcytometery, IHC-Immunohistochemistry, LN Bx- Lymph node biopsy, DFS/EFS- Disease free survival/ Event free survival,						

Marshall et al discovered that Hodgkin lymphoma tumor infiltrating lymphocytes are rich in Treg cell type. These cells induce a profoundly immunosuppressive environment responsible for ineffective immune clearance in HL.64 Similar findings were found by Schrek et al who found that Classic Hodgkin lymphoma microenvironment is rich in Th-2 and Treg.65

Alvaro et al showed that increased infiltration of diseased lymph node in HL is associated with a positive prognostic significance and improved overall survival.18

We found that only a single study was carried out to find out the frequency of in peripheral blood of HL patient by E. Guduz et

al, which showed that increased Treg frequency is associated with poor prognosis. 66

Conclusion

Tregs have a pivotal role in maintaining the immune system in healthy individuals. In solid cancer and in hematological malignancies, Tregs show a major immunosuppressive activity, thus playing a fundamental role in tumor cell growth, proliferation, and survival. Many articles Published on the prognostic relevance of the Treg number in hematological malignancies show conflicting results. In our opinion, this is most likely due to the difference in the experimental approaches that are used. In fact, dissimilar tissues have been examined (i.e., peripheral blood, bone marrow, and lymph node) and different analytic methodologies have been used (i.e., flow cytometry versus Immunohistochemistry). Moreover, while most of the studies done on whole blood compartment; others examined the Treg population in isolated peripheral blood mononuclear cells. This is of prime importance to stress the need to apply standardized process in the study of Tregs in hematological malignancies and in cancer in general.

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