

## The Role of T Regulatory Lymphocytes in Lymphoma

Ahmad Nasser<sup>\*,\*\*\*</sup>, Afra Khosravi<sup>\*\*</sup>, Reza Azizian<sup>\*</sup>

<sup>\*</sup>Student Research Committee, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

<sup>\*\*</sup>Immunology Department, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

<sup>\*\*\*</sup>Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran

### Abstract

T regulatory cells play a crucial role in immunological unresponsiveness to self-antigens and in suppressing excessive immune responses deleterious to the host. T regulatory cells are produced in the thymus as a functionally mature subpopulation of T cells. They can be induced from naive T cells in the periphery and express their marker as a forkhead/winged helix transcription factor called FoxP3. In patients with lymphomas where T regulatory cells serve as suppressor anti-tumor cytotoxicity, decreased numbers of T regulatory cells are associated with a favorable prognosis. In contrast, in patients with lymphomas where T regulatory cells function as anti-tumor cytotoxic agents, enhanced numbers of T regulatory cells are associated with a favorable prognosis.

Tumors actively promote the accumulation of these cells through several mechanisms that involve activation of naturally occurring T regulatory cells as well as conversion of non-T regulatory cells into T regulatory cells. Tumor-derived prostaglandin E2 can increase T regulatory cell activity and induce a regulatory phenotype in CD4<sup>+</sup>CD25<sup>+</sup>T cells. On the other hand, a balance between T regulatory and Th17 cells is essential for maintaining homeostasis of anti-tumor immunity. Accelerating processes such as increasing the amounts of IL-6 or IL-17 can enhance FoxP3 T regulatory cell expression and result in a lymphoma or inactivation of T cell CD4<sup>+</sup>. This effect is the reason for malignancy and a reduction in anti-tumor immune response. In this systematic review we intend to analyze this relationship. We have collected and analyzed the majority of recently published articles on the role of T regulatory cells as a review article.

**Keywords:** Malignancy, FoxP3, Treg, Lymphoma

#### Corresponding Author:

Afra Khosravi, PhD  
Immunology Department,  
Faculty of Medicine, Ilam  
University of Medical Sciences  
Ilam, Iran  
Tel: +98-841-334-8495  
Email: afrakhosravi@yahoo.co.uk

### Introduction

Many studies after the identification of CD4<sup>+</sup>CD25<sup>+</sup>T cells have focused on the role of

immunoregulatory T cells in the suppression of anti-tumor responses.<sup>1</sup> Natural T regulatory (Tregs) cells arise in the thymus but some Tregs

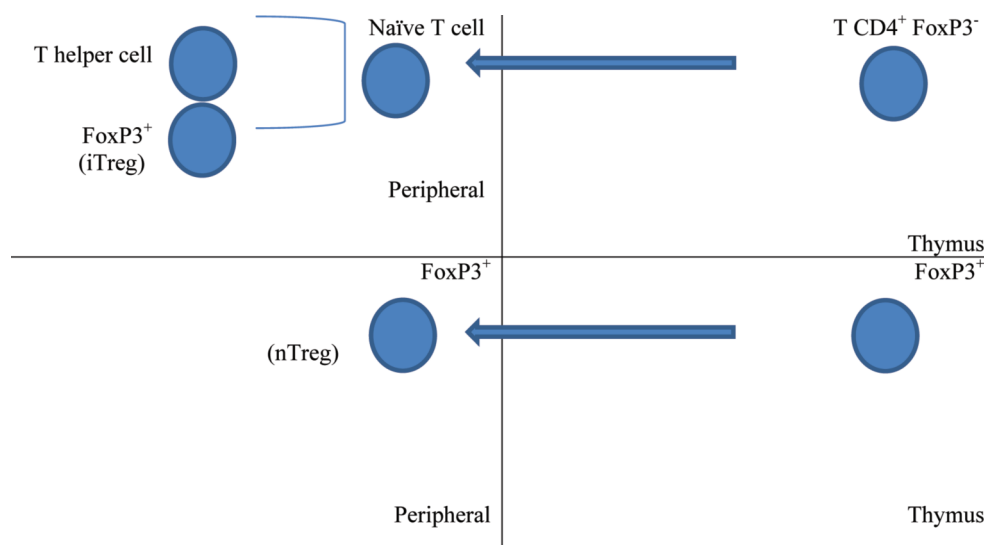
may also be induced in the periphery,<sup>2</sup> expressing their marker as a forkhead/winged helix transcription factor called FoxP3<sup>3</sup> and mediate the peripheral tolerance by suppressing the level of autoreactive T cells.<sup>4</sup> T regulatory cells also inhibit autoimmune and inflammatory diseases as well as anti-tumor immune responses.<sup>5-7</sup> An increased number of Tregs have been observed in different malignancies.<sup>8</sup> T regulatory cells have numerous markers. Up-regulation of markers such as CD25, CTLA-4, and the glucocorticoid-induced TNFR family-related gene (GITR) induce Tregs to inhibit the activities of other cells such as TCD4<sup>+</sup> cells.

The development of a clinically efficient cancer immunotherapy has been problematic and mostly attributed to the suppressed T cell function described in many cancer patients. Inhibition of anti-tumor immune responses is mainly linked to inhibitory factors (Tregs) present in cancer patients.<sup>9</sup> Patients with Chronic lymphocytic leukemia (CLL) who undergo chemotherapy with fludarabine, cyclophosphamide or alemtuzumab have altered ratios of CD4<sup>+</sup> to CD8<sup>+</sup> T cells. These chemotherapeutic agents are cytotoxic for T cells. This effect is attributed to fludarabine which causes increased susceptibility of CD8<sup>+</sup> T cells to apoptosis compared to CD4<sup>+</sup> T cells.<sup>10</sup> The

use of low-dose cyclophosphamides has been shown to decrease the number of Treg cells in murine models.<sup>11</sup> An increase in the frequency of Tregs was found in untreated patients with intermediate (Binet B) or extended disease (Binet C) CLL according to the Binet clinical staging system. Patients treated with chemotherapy regimens that contained fludarabine showed significantly reduced amounts of Tregs.<sup>12</sup> In a murine fibrosarcoma model, the Tregs observed in the tumor environment were the majority of tumor-infiltrating lymphocytes (TILs) at the late stage of tumor progression. The evacuation of Tregs during the effector rather than priming phase successfully enhanced anti-tumor immunity.<sup>13</sup> As can be seen from the above data, numerous studies have been performed regarding the role of Tregs in cancer and autoimmune diseases. When Tregs are present in tissue, a defect in the immune response can be seen. This review is a systematic review on the results of different studies published thus far about the role of Tregs in different lymphomas.

### *T regulatory (Treg) cells*

T regulatory cells are classified into two groups, natural and adaptive (induced). Natural Tregs (nTregs) are CD4<sup>+</sup>CD25<sup>+</sup> T cells which develop



**Figure 1.** Differentiation of naive CD4<sup>+</sup> T cells into T regulatory (Tregs) cells. FoxP3 Tregs in peripheral tissues can be divided into naturally occurring Tregs (nTregs) and adaptive/inducible Tregs (iTregs). Naturally occurring Tregs differentiate in the thymus, whereas iTregs are generated from naive T cells in the periphery.

**Table 1.** Markers of T regulatory (Treg) cells.

| Marker         | Author                        | Study title                                                                                                                                                                                                      |
|----------------|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| FoxP3          | Hori S, Nomura T, Sakaguchi S | Control of regulatory T cell development by the transcription factor FoxP3                                                                                                                                       |
|                | Gavin MA, Torgerson TR        | Single-cell analysis of normal and FoxP3-mutant human T cells: FoxP3 expression without regulatory T cell development                                                                                            |
|                | Morgan ME, van Bilsen JH      | Expression of FoxP3 mRNA is not confined to CD4 <sup>+</sup> CD25 <sup>+</sup> Treg cells in humans                                                                                                              |
|                | Wang J, Ioan-Facsinay A       | Transient expression of FoxP3 in human activated nonregulatory CD4 <sup>+</sup> T cells                                                                                                                          |
| CD25           | Lawson JM, Tremble J          | Increased resistance to CD4 <sup>+</sup> CD25 <sup>hi</sup> regulatory T cell-mediated suppression in patients with type 1 diabetes                                                                              |
|                | Beavis PA                     | Resistance to regulatory T cell-mediated suppression in rheumatoid arthritis can be bypassed by ectopic FoxP3 expression in pathogenic synovial T cells                                                          |
|                | Bodor J                       | Regulatory T cell-mediated suppression: Potential role of ICER                                                                                                                                                   |
| CTLA-4 (cd152) | Tang Q                        | Distinct roles of CTLA-4 and TGF-beta in CD4 <sup>+</sup> CD25 <sup>+</sup> regulatory T cell function                                                                                                           |
|                | Sakaguchi S                   | Regulatory T cells: How do they suppress immune responses?                                                                                                                                                       |
|                | Davidson TS                   | Polyclonal Treg cells modulate T effector cell trafficking                                                                                                                                                       |
|                | Kolar P                       | CTLA-4 (CD152) controls homeostasis and suppressive capacity of regulatory T cells in mice                                                                                                                       |
| ICOS           | Ito T                         | Two functional subsets of FoxP3 <sup>+</sup> regulatory T cells in human thymus and periphery                                                                                                                    |
|                | Redpath SA                    | ICOS controls FoxP3 <sup>+</sup> regulatory T cell expansion maintenance and IL-10 production during helminth infection                                                                                          |
| CD39           | Deaglio S                     | Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression                                                                                                      |
|                | Kobie JJ, Shah P              | Treg and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5-adenosine monophosphate to adenosine                                                                 |
| CD122          | Agustina TE                   | CD8 <sup>+</sup> CD122 <sup>+</sup> regulatory T cells (Tregs) and CD4 <sup>+</sup> Tregs cooperatively prevent and cure CD4 <sup>+</sup> cell-induced colitis                                                   |
| CD132          | Ohki S                        | CD8CD122 T cells, a newly identified regulatory T subset, negatively regulate Graves' hyperthyroidism in a murine model                                                                                          |
| CD127 (IL-7Ra) | Banham AH                     | Role of regulatory T cells in human diseases Chatila TA                                                                                                                                                          |
|                | Weihong L                     | Cell-surface IL-7 receptor expression facilitates the purification of FoxP3 regulatory T cells<br>CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4 <sup>+</sup> Treg cells |

and migrate from the thymus to perform their role in immune homeostasis. Induced Tregs (iTregs) are non-regulatory CD4<sup>+</sup> T cells which acquire CD25 (IL-2R  $\alpha$ ) expression outside of the thymus and are typically induced by inflammation and disease processes such as cancer

(Figure 1).<sup>14</sup> To date, no specific marker can distinguish nTregs from iTregs. Recently, reports state that the Helios family may be helpful for distinguishing nTregs from iTregs, however others have reported that Helios is also highly expressed

on Th2 and T follicular helper cells.<sup>15</sup>

Different specificities for CD4<sup>+</sup>CD25<sup>high</sup> FoxP3<sup>+</sup> Treg cells have been described such as their ability to actively inhibit CD4<sup>+</sup>CD25<sup>+</sup> T cells, CD8<sup>+</sup> T cells, dendritic cells (DCs), natural killer (NK) cells,<sup>1</sup> natural killer T (NKT) cells, and B cells via cell-to-cell contact and dose-dependent mechanisms.<sup>17,18</sup>

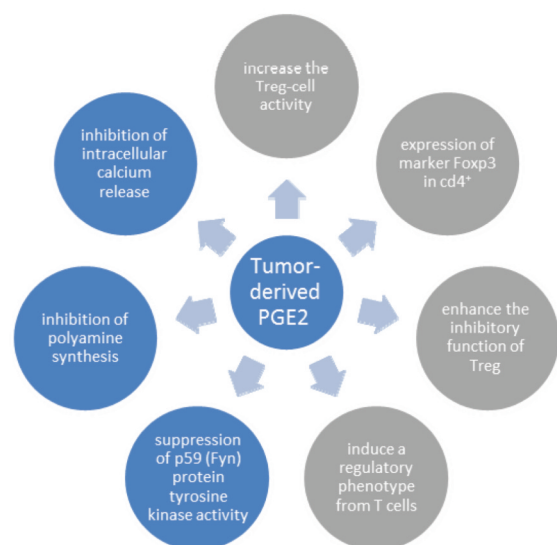
FoxP3, a member of the forkhead transcriptional-factor family, is the major regulator and suppressive function of Treg cells.<sup>19</sup> Retroviral transduction of FoxP3 into CD4<sup>+</sup>CD25<sup>+</sup> T cells results in the acquisition of TCR hyporesponsiveness, up-regulation of Treg associated markers (Table 1) such as CD25, CTLA<sup>+</sup>, GITR, and the ability to suppress the activation of effector T cells in vivo and in vitro, all of which suggest that FoxP3 may be sufficient to affect the Treg developmental program efficiently.<sup>20</sup> Treg cells have many unique specifications that include expression of high levels of the CD257 marker though there are another marker such as CD62L<sup>+</sup>, ICOS<sup>+</sup>, and CD127<sup>low</sup> for Tregs. Tregs are hyporesponsive to T cell receptor (TCR) stimulation in vitro and are unable to proliferate or produce activation-induced cytokines such as IL-2 or IFN $\gamma$ .<sup>21</sup> Multiple protein-protein interactions that involve several different domains have been suggested to be engaged by FoxP3. IPEX syndrome results from loss of function by mutations in the FoxP3 gene.<sup>22</sup> The FKH domain is a hotspot for mutation in humans with IPEX that is usually required for FoxP3 to bind to DNA and localize to the nucleus<sup>23</sup> in addition to mediating a direct interaction with the transcription factor NFAT. The protein product of FoxP3 is approximately 431 amino acids in length and has 198 amino acids in the amino terminal region. It is non-conserved, has a C2H2 zinc finger, a leucine zipper, and a winged-helix/forkhead (FKH) domain at its carboxyl-terminus.<sup>24</sup>

### Lymphomas

There are three major categories of lymphoid malignancies, B cell, T cell and Hodgkin's

lymphoma (HL).<sup>25</sup> B cell lymphomas are more common than T cell lymphomas. About 90% people diagnosed with non-Hodgkin's lymphoma (NHL) have a B cell lymphoma. The most frequent B cell lymphomas are diffuse large B cell lymphoma and follicular lymphoma. The less common types include extra-nodal marginal zone lymphoma of mucosa-associated tissue (MALT), mantle cell, Burkett's lymphoma, mediastinal large B cell, nodal marginal zone lymphoma, small lymphocytic lymphoma (SLL) and lymphoplasmacytic lymphoma which is also called Waldenstrom's macroglobulinemia. Diffuse large B cell lymphoma (DLBCL) has not been defined in subgroups according to morphology due to discrepancies arising from inter- and intra-observer reproducibility.<sup>26</sup> The rearranged immunoglobulin genes in DLBCL carry mutations that are important in hypermutation and diversification of antibody that normally occurs only within the germinal center of secondary lymphoid organs.<sup>27</sup>

T cell lymphomas include peripheral T cell lymphoma, and cutaneous lymphomas such as Mycosis fungoides and Sezary syndrome, anaplastic large cell lymphoma and lymphoblastic lymphoma which is mainly a T cell lymphoma, however it can also be a B cell lymphoma.



**Figure 2.** Prostaglandin E2 (PGE2) and induced immune-suppression (blue circle). T regulatory (Treg) cell activity (grey circle).

**Table 2.** The variations of Tregs in different malignancies.

| Result                  | Effect                                                 | Disease                                                                                   |
|-------------------------|--------------------------------------------------------|-------------------------------------------------------------------------------------------|
| Tumor                   | Secret PGE-2                                           | Increase T regulatory (Treg) cell activity<br>expression of FoxP3 inhibits CD3 activation |
| Malignant B cells       | High expression of B7-H1                               | Conversion of Tcd4 <sup>+</sup> to Treg cells                                             |
| Malignant T cells       | Interaction of T cells with immature dendritic cell DC | Shows all traits of Treg cells                                                            |
| Hodgkin's lymphoma (HL) | Express and secrete mediators of Th2 and Treg          | Suppresses the immune response                                                            |

Cutaneous T cell lymphoma (CTCL) is a clonal malignancy of CD4<sup>+</sup> helper T cells that express a memory phenotype and the propensity to accumulate in the epidermis in proximity to Langerhans cells (LCs) which are immature DCs.<sup>28-33</sup> It has been suggested that LCs are stimulated by LC-mediated antigen presentation which leads to the hypothesis that CTCL is a disease of chronic antigen stimulation whose growth is promoted by LC presentation of virally, bacterially, or chemically modified peptides.<sup>34,35</sup>

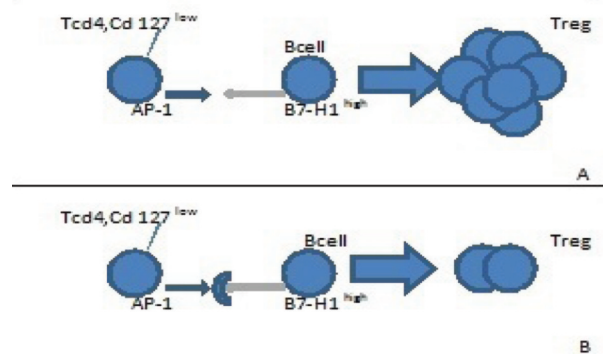
Viruses or other infectious agents have long been considered as the etiologic agents for HL. Epstein-Barr virus is the main candidate as this infectious agent has a unique and distinct history, epidemiology, treatment, and biology. Recently, autoimmune processes have suggested a potential role for the immune-related and inflammatory conditions in the etiology and pathogenesis of HL.<sup>36</sup> The Rye classification scheme for HL is based on the histologic subtypes that represent morphologic variations of a neoplasm in which Hodgkin and Reed-Sternberg (HRS) cells are embedded in a reactive background and show a characteristic cellular composition for each histological type.<sup>37</sup>

*T regulatory (Treg) and natural T regulatory (nTreg) cell trafficking at steady state*

Cellular recruitment to the tissue is related to local induction of cytokines and chemokines, which are crucial regulators of immune cell trafficking. CD4<sup>+</sup> T cells are divided into Treg cells and conventional T helper (Th) cells. The Th cells control adaptive immunity against pathogens and cancer by activating other effector immune cells.

T regulatory cells are defined as CD4<sup>+</sup> T cells in charge of suppressing potentially deleterious activities of Th cells.<sup>38</sup> CD4<sup>+</sup>CD25<sup>high</sup> FoxP3<sup>+</sup> Tregs can suppress other immune cells and are critical mediators of peripheral self-tolerance.<sup>39,40</sup> Populations of CD3<sup>+</sup>CD4<sup>+</sup> T cells that inhibit effector B and T cells can produce immunosuppressive cytokines such as IL-10 and TGFβ. They have a major role in suppressing anti-self-immune responses to prevent autoimmune diseases.

The high percentage of nTreg cells with effector/memory phenotype and tissue homing receptor expression in adults enables them to migrate from blood into peripheral tissues. Natural T regulatory cell entry into tissues at a steady state may enable a quick and effective suppressive response against unwanted local immune responses. Their exit from tissues prevents accumulation that could lead to destructive immune responses against pathogens or tumors.



**Figure 3.** A. Interaction of B7-H1 high lymphoma B cell with AP-1 of Tcd4<sup>+</sup> can produce Treg cells from Tcd4<sup>+</sup> cells. B. Blocking the interaction of B7-H1 and AP-1 using antibody results in reduced generation of Treg cells.

After leaving the thymus, naive T cells circulate in the blood from which they migrate into secondary lymphoid tissues due to expression of L-selectin (CD62L) and the chemokine CC receptor (CCR)7.<sup>41</sup>

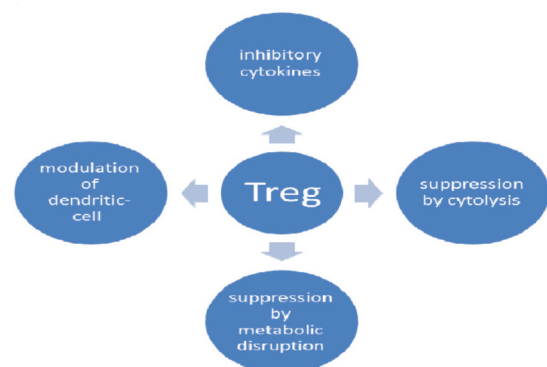
In adoptive transfer experiments, Tregs with CCR7<sup>-</sup>, CD62L<sup>-</sup> fail to achieve lymph node directed homing and thus, to suppress proliferation of CD4<sup>+</sup> naive T cells.<sup>2</sup> The majority of the nTreg cells in cord blood express the gut-homing integrin  $\alpha 4\beta 7$  and only a minor population of cells expresses CCR4, whereas in adult nTreg cells, the majority express CCR4 but not  $\alpha 4\beta 7$ . Switch expression of nTreg homing receptors from  $\alpha 4\beta 7$  to CCR4 occurs between 1.5 and 3 years of age, along with a change from the CD45RA<sup>+</sup> naive to the CD45RO<sup>+</sup> memory phenotype. These conversions indicate a crucial role for the gut in nTreg cell stimulation by exogenous antigens in early stages of life.<sup>43</sup> There are at least two populations of FoxP3<sup>+</sup>Treg cells in human adult blood, CD45RA<sup>+</sup> naive phenotype and CD45RO<sup>+</sup> memory phenotype. The FoxP3<sup>+</sup>Treg cells with the CD45RA<sup>+</sup> naive phenotype represent the majority in human cord blood.<sup>44</sup> To distinguish between these populations of Treg cells, CD95 can be used. Memory Treg cells are sensitive to induce apoptosis with CD95 but naive Treg cells are resistant.<sup>13</sup>

Kaede transgenic mice are engineered to express the photo-convertible fluorescence protein Kaede, which changes from green to red when exposed to violet light. In these mice during a cutaneous immune reaction, the frequency of UV-exposed migratory CD4<sup>+</sup> T cells increases in draining lymph nodes by ten-fold compared to the steady state. Approximately half of the migrating CD4<sup>+</sup> T cells are Treg cells, hence this effect shows that Treg cells protect the skin from autoimmune responses.<sup>45</sup> Although Treg cells migrate into both inflammatory sites and draining lymph nodes during an immune response, they have different functions in each location. This event suggests that Treg cells in draining lymph nodes and inflammatory sites may not simply be

a division of function, but more Treg cells migrate between peripheral tissues and draining lymph nodes.<sup>46</sup> Maintenance of Tregs in the periphery is aided by signals via interleukin-2 (IL-2), CD28 and transforming growth factor- $\beta$  (TGF- $\beta$ ).<sup>47</sup>

#### *Tumors and conversion of non-T regulatory (Treg) cells into Treg cells*

Tumors actively promote the accumulation of Treg cells through several mechanisms that involve activation of naturally occurring Treg cells as well as conversion of non-Treg cells into Treg cells.<sup>48</sup> Tumor-derived prostaglandin E2 (PGE2) can increase Treg cell activity. Expression of the marker FoxP3,<sup>49</sup> enhances the *in vitro* inhibitory function of human Treg cells and induces a regulatory phenotype in CD4<sup>+</sup>CD25<sup>+</sup>T cells.<sup>50</sup> Studies have shown that purified CD4<sup>+</sup>CD25<sup>+</sup> Treg cells have significant enhancement of their inhibitory function after incubation with PGE2 for 24 hours *in vitro*<sup>51</sup> and pre-incubation of CD4<sup>+</sup>CD25<sup>-</sup> T cells with PGE2 allows the regulatory T cell function, the PGE2-treated CD4<sup>+</sup>CD25<sup>-</sup> stimulator cells have been shown to inhibit CD3 activated proliferation of autologous CD4<sup>+</sup>CD25<sup>-</sup> responder T cells. Accelerated levels of intracellular cAMP were associated with reduced IL-2 production;<sup>52</sup> cAMP causes elevating agonists like PGE2 to induce FoxP3 gene expression. Other pathways of PGE2-induced immune-suppression include inhibition of intracellular calcium release, inhibition of polyamine synthesis, and suppression of p59



**Figure 4.** Mechanism of T cell regulation.

(Fyn) protein tyrosine kinase activity (Figure 2).<sup>53</sup> These Treg cells have been shown to secrete TGF- $\beta$ , which provided the first evidence that Treg cells contribute to immune dysfunction in patients with cancer.<sup>54</sup> The major role of TGF- $\beta$  in immune regulation is to suppress the immune response.<sup>55</sup> TGF- $\beta$  controls T cell response through Treg-dependent and -independent mechanisms.

#### *Role of malignant B cells in the conversion of non-T regulatory (Treg) cells to Treg cells*

Tumors may induce immunologic tolerance by promoting the expansion, recruitment and activation of Treg cells.<sup>56</sup> Treg cells, which account for approximately 5% to 10% of peripheral CD4<sup>+</sup> T cells in both mice and humans include naturally occurring CD4<sup>+</sup>CD25<sup>+</sup> Treg cells as well as peripherally induced CD4<sup>+</sup> Tregs.<sup>57</sup> Treg cells express lower levels of CD127 than other CD4<sup>+</sup> T cells and CD127 expression reverse correlates with FoxP3 expression.<sup>58-60</sup>

Several studies have shown that increased frequencies of CD4<sup>+</sup>CD25<sup>+</sup> Treg cells in peripheral blood and LN mediated severe suppressive activity compared to those from healthy individuals.<sup>61,62</sup> Patients with aggressive B cell NHL had higher frequencies of Treg cells in peripheral blood than patients with indolent B cell NHL.<sup>63</sup> Yixiang Han et al. have found significant induction of FoxP3<sup>+</sup> cells from CD4<sup>+</sup>CD25<sup>-</sup> T cells in the presence of malignant B cells. In addition, FoxP3<sup>+</sup> cells could not be induced from CD4<sup>+</sup> CD25<sup>-</sup> T cells in the presence of normal B cells from healthy persons. B7-H1, a newly discovered member of the B7 family, is expressed on B cell, T cell, monocytes and DCs. Some studies have shown higher expression of B7-H1 in lymphoma B cells than normal B cells. Programmed death 1 (PD-1) is a member of the CD28/CTLA-4 family which has a major role in the maintenance of peripheral tolerance. PD-1 is a receptor of B7-H1;<sup>64</sup> blocking the interaction of PD-1 and B7-H1 by the anti-B7-H1 antibody could partly inhibit induction of the CD4<sup>+</sup> FoxP3<sup>+</sup> phenotype in some patients (Figure 3). Blocking the

- PD-1/ B7-H1 pathway could reduce the generation of Treg cells, suggesting that PD-1/B7-H1 signaling may regulate the generation of Treg cells in B cell NHL.

#### *Role of T cell lymphoma in conversion of non-T regulatory (Treg) cells to Treg cells*

Cutaneous T cell lymphoma cells (CTCL) are a clonal malignant form of CD4<sup>+</sup> Th cells that express a memory phenotype with the tendency to accumulate in the epidermis in the proximity of Langerhans cells. For in vitro purposes the proliferation of CTCL and autologous myeloid DCs requires direct contact between these two cell populations. Only immature autologous DCs support CTCL cell replication and CTCL cells in contrast produce interleukin-10 (IL-10), which can maintain long-term DC immaturity.<sup>65,66</sup> Cutaneous T cell lymphoma cells retain the phenotype and genotype of the primary malignant clone, whereas APCs have both immature and mature DCs. The malignant cells initially proliferate in the epidermis and later escape from the skin, spreading into the blood and other organs.<sup>67</sup> Some studies have shown that mitogen, antigen, and mixed leukocyte cultures with or without cytokines only minimally stimulate proliferation of isolated CTCL cells.<sup>68,69</sup> Cutaneous T cell lymphoma cells cultured with IL-2 and IL-7 and DCs cultured with granulocyte monocyte colony-stimulating factor (GM-CSF) and IL-4 did not survive and proliferate within few weeks after culturing.<sup>70</sup> Berger et al. have reported that offering immature DCs with apoptotic CTCL cells led to the expression of T Cell Receptor in malignant T cells. The Cutaneous T cell lymphomas respond by proliferated and up-regulated cytoplasmic cytotoxic T lymphocyte antigen-4 (CTLA-4), FoxP3, and membrane CD25, and secreted IL-10 as a immunosuppressive cytokine and transforming growth factor-(TGF- $\beta$ ) that all are the features of T-regulatory (Treg) cells.<sup>71</sup> This phenomenon indicates the relationship between immature DCs and CTCLs that can produce Treg cells from CTCL cells.

### *Hodgkin's lymphoma (HL) and T regulatory (Treg) cells*

Cytotoxic T lymphocytes (CTL) and Th1 cells are effective mediators of anti-tumor immunity but Th2 cells may contribute to tumor growth.<sup>72</sup> Hodgkin and Reed-Sternberg (HRS) cells express and secrete mediators for attracting the Th2 and immunosuppressive Treg that have been identified in classic HL tissue.<sup>73,74</sup>

Studies have shown that expression of T cell-specific tissue factor (TF) in HRS cells would allow the production of T cell-specific cytokines, thus facilitating autocrine stimulation of tumor cell growth.<sup>75</sup> Treg cells not only suppress tumor-specific T cells but can also directly suppress B lymphocytes, which together with Th2 cells are central mediators of the humoral immune response.<sup>76,77</sup> In a pioneering report, Curiel et al. have demonstrated a correlation between intratumoral Treg cells and poor survival in ovarian cancer which showed the detrimental effect of suppressor T cells in disease outcome.

Th2 cells, by the activation of B cells or the production of the immunosuppressive cytokine IL-10, are associated with aggressive tumors. However this is not a general phenomenon, as these cells are also associated with favorable outcomes in HL and breast cancer which suggests a protective effect of antibodies in these diseases.<sup>78,79</sup> In other words Th1 cells and the cytokines such as IFN $\gamma$  are associated with good clinical outcome for many cancer types. Thus, general characteristics appear in which cytotoxic T cells, memory T cells and Th1 cells are likely associated with prolonged survival whereas the differential effect of other Th cell populations and Treg cells may be attributed to their bad prognoses.

### *T helper 17 (Th17) and T regulatory (Treg) cells*

T helper 17 (Th17) cells, a recently described CD4<sup>+</sup> T cell subset, protect hosts against parasitic and fungal infections and interfere in inflammatory reactions and autoimmunity. The balance between Treg and Th17 cells is particularly essential for

maintaining homeostasis of anti-tumor immunity.<sup>80</sup> Th17 cells differentiate in the presence of IL-6, IL-1, IL-21, IL-23 with TGF- $\beta$  and produce IL-17 and IL-22.<sup>81</sup>

IL-17 induces expression of a number of chemokines and cytokines that include IL-6, TGF- $\beta$ , G-CSF or GM-CSF, matrix metalloproteinase and ICAM-1 in a variety of cell types, including bone marrow stromal cells.<sup>82</sup> Treg and Th17 developmental programs are equally interconnected. Following TCR stimulation a naive T cell can express FoxP3 and become a Treg cell in the presence of TGF- $\beta$  while in the presence of TGF- $\beta$  and IL-6 or IL-21, the Treg expansion pathway is abrogated, and T cells develop into Th17 cells. Only the combination of TGF- $\beta$  with IL-6 or IL-21, but neither of them alone, induces a huge production of IL-17 by naive T cells.<sup>44,83</sup> Therefore, IL-6 plays a pivotal role in dictating the balance between the generation of Tregs and Th17 cells.<sup>14</sup> IL-6, an acute phase protein that induces during inflammation, inhibits the generation of FoxP3<sup>+</sup> Treg cells that are induced by TGF- $\beta$  during inflammation.<sup>22</sup> The mechanism by which IL-6 and IL-21 act as switch factors relies on the control of the FoxP3/ROR $\gamma$ t balance.<sup>83</sup> A low frequency of TH17 cells with no detectable amount of IL-17 producing cells present in the tumor microenvironment can be seen. The absence of B lymphoma cells can occur with treatment by IL-1 $\beta$ /IL-6 or lipopolysaccharide (LPS) which in turn enhances IL-17 expression in CD4<sup>+</sup> T cells. This enhancement is attenuated when CD4<sup>+</sup> T cells and lymphoma B cells are cultured together.

### *Mechanism of function of T cell regulation*

According to the cross regulation model proposed by Leon et al., suppression by Treg cells is antigen specific.<sup>84</sup> In this model that has received experimental support, Treg cells are proposed to be autoreactive and suppress Th cells that have the same antigen specificity.<sup>85,86</sup>

From a functional perspective, the various potential suppression mechanisms of Treg cells can be grouped into four basic modes of action:



suppression by cytolysis, suppression by inhibitory cytokines, suppression by modulation of DC maturation or function and suppression by metabolic disruption (Figure 4).

Most studies show that Treg cells mediate suppression by inhibiting the induction of IL-2 mRNA and mRNA for other effector cytokines in the responder FoxP3<sup>-</sup> T cells.<sup>88</sup>

The role of IL-2 utilization in the suppressive mechanism of Treg cells is under dispute. Treg cells express all three markers of the high-affinity IL-2R (CD25), CD122, and CD132. IL-2 is essential for Treg cell homeostasis in vivo and important for their efficient suppressor function *in vitro*.<sup>88,89</sup>

## Conclusion

Recent studies in other cancers have suggested that Treg cells are involved in the control of anti-tumor immunity by inducing tolerance to the tumor. It has been shown that Treg cells influence tumor immune responses by suppressing tumor-specific immune cells. However, scant data exists regarding the effect of Treg cells on tumor-specific T cell immunity in B cell NHL and subsequently on malignant B cell growth.

Anti-tumor response in B cell NHL is profoundly suppressed by the presence of large numbers of intratumoral Treg cells. NHL B cells induce FoxP3 expression in CD4<sup>+</sup>CD25<sup>-</sup> T cells and contribute to the development of Treg cells in malignant lymph nodes. Accelerating processes such as increasing the amount of Treg cells (e.g., increasing the amount of the IL-6 or IL-17) can enhance FoxP3 Treg expression which results in lymphoma production or inactivation of T cell CD4<sup>+</sup>; this effect is the reason behind the malignancy of B cells and reduction in anti-tumor immune responses. T helper 17 causes autoimmune diseases whereas Treg cells suppress autoimmune diseases and anti-tumor immune responses. In multiple myeloma, conversion of Treg cells to Th17 mediated by cytokines or other mediators have shown some therapeutic effects on such patients. It can be seen from different data that Treg cells play an important role in

lymphomas, however the mechanisms of action warrant additional studies.

## Conflict of Interest

No conflict of interest is declared.

## References

1. Scaglione BJ, Salerno E, Gala K, Pan M, Langer JA, Mostowski HS, et al. Regulatory T cells as central regulators of both autoimmunity and B cell malignancy in New Zealand Black mice. *J Autoimmun.* 2009; 32(1): 14-23.
2. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immunotolerance. *Cell.* 2008;133(5): 775-87.
3. Ni Choileain N, Redmond HP. Regulatory T-cells and autoimmunity. *J Surg Res.* 2006; 130(1):124-35.
4. Longhi MS, Hussain MJ, Mitry RR, Arora SK, Mieli-Vergani G, Vergani D, et al. Functional study of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells in health and autoimmune hepatitis. *J Immunol.* 2006; 176(7): 4484-91.
5. Shimizu J, Yamazaki S, Sakaguchi S. Induction of tumor immunity by removing CD25<sup>+</sup>CD4<sup>+</sup> T cells: a common basis between tumor immunity and autoimmunity. *J Immunol.* 1999; 163:5211-8.
6. Ghiringhelli F, Ménard C, Terme M, Flament C, Taieb J, Chaput N, et al. CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner. *J Exp Med.* 2005; 202(8): 1075-85.
7. Takahashi T, Sakaguchi S. Naturally arising CD25<sup>+</sup>CD4<sup>+</sup> regulatory T cells in maintaining immunologic self-tolerance and preventing autoimmune disease. *Curr Mol Med.* 2003;3: 693-706.
8. Marshall NA, Christie LE, Munro LR, Culligan DJ, Johnston PW, Barker RN, et al., Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood.* 2004; 103(5): 1755-62.
9. Beyer M, Kochanek M, Darabi K, Popov A, Jensen M, Endl E, et al. Reduced frequencies and suppressive function of CD4<sup>+</sup> CD25<sup>+</sup> high regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. *Blood.* 2005; 106(6): 2018-25.
10. Gamberale R, Galmarini CM, Fernández-Calotti P, Jordheim L, Sánchez-Avalos J, Dumontet C, et al. In vitro susceptibility of CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets to fludarabine. *Biochem Pharmacol.* 2003; 66(11): 2185-91.
11. Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, Sabzevari H. Inhibition of CD4<sup>+</sup>25<sup>+</sup>T

- regulatory cell function implicated in enhanced immune response by low dose cyclophosphamide. *Blood*. 2005; 105(7): 2862-8.
12. Beyer M, Kochanek M, Darabi K, Popov A, Jensen M, Endl E, et al. Reduced frequencies and suppressive function of CD4<sup>+</sup>CD25<sup>hi</sup> regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. *Blood*. 2005; 106(6): 2018-25
  13. Krammer PH, Arnold R, Lavrik IN. Life and death in peripheral T cells. *Nat Rev Immunol*. 2007; 7(7):532-42.
  14. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3<sup>β</sup> regulatory T cells: More of the same or a division of labor? *Immunity*. 2009; 30(5): 626-35.
  15. Serre K, Bénézech C, Desanti G, Bobat S, Toellner KM, Bird R, et al. Helios is associated with CD4 T cells differentiating to T helper 2 and follicular helper T cells in vivo independently of Foxp3 expression. *PLoS One*. 2011; 6(6):e20731.
  16. Ghiringhelli F, Ménard C, Terme M, Flament C, Taieb J, Chaput N, et al. CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells inhibit natural killer cell functions in a transforming growth factor- $\beta$ -dependent manner. *J Exp Med*. 2005; 202(8):1075-85.
  17. Lim HW, Hillsamer P, Banham AH, Kim CH. Cutting edge: direct suppression of B cells by CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells. *J Immunol*. 2005. 175(7): 4180-3.
  18. Ralainirina N, Poli A, Michel T, Poos L, Andrès E, Hentges F, et al. Control of NK cell functions by CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells. *J Leukoc Biol*. 2007; 81 (1): 144-53.
  19. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science*. 2003; 299(5609): 1057-61.
  20. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells. *Nat Immunol*. 2003; 4(4):330-6.
  21. Schubert LA, Jeffery E, Zhang Y, Ramsdell F, Ziegler SF. Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J Biol Chem*. 2001; 276(40):37672-9.
  22. Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: Forkhead box protein 3 mutations and lack of regulatory T cells. *J Allergy Clin Immunol* 2007; 120(4):744-50.
  23. Lopes JE, Torgerson TR, Schubert LA, Anover SD, Ocheltree EL, Ochs HD, et al. Analysis of FOXP3 reveals multiple domains required for its function as a transcriptional repressor. *J Immunol*. 2006; 177(5): 3133-42.
  24. Brunkow ME, Jeffery EW, Hjerrild KA, Paepfer B, Clark LB, Yasayko SA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet*. 2001;27(1):68-73.
  25. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994; 84(5):1361-92.
  26. Rosenberg SA. Classification of lymphoid neoplasms. *Blood*. 1994; 84(5):1359-60.
  27. Klein U, Goossens T, Fischer M, Kanzler H, Braeuning A, Rajewsky K, et al. Somatic hypermutation in normal and transformed human B cells. *Immunol Rev*. 1998; 162:261-80.
  28. Broder S, Edelson RL, Lutzner MA, Nelson DL, MacDermott RP, Durm ME, et al. The Se'zary syndrome: a malignant proliferation of helper T cells. *J Clin Invest*. 1976; 58(6):1297-306.
  29. Berger CL, Warburton D, Raafat J, LoGerfo P, Edelson RL. Cutaneous T cell lymphoma: neoplasm of T cells with helper activity. *Blood*. 1979; 53(4):642-51.
  30. Picker LJ, Michie SA, Rott LS, Butcher EC. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am J Pathol*. 1990; 136(5):1053-68.
  31. Edelson RL, Berger CL, Raafat J, Warburton D. Karyotype studies of cutaneous T cell lymphoma: evidence for clonal origin. *J Invest Dermatol*. 1979; 73(6):548-50.
  32. Rowden G, Phillips TM, Lewis MG, Wilkinson RD. Target role of Langerhans cells in mycosis fungoides: transmission and immuno-electron microscopic studies. *J Cutan Pathol*. 1979; 6(5):364-82.
  33. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. *Annu Rev Immunol*. 2000; 18:767-811.
  34. Tan RS, Butterworth CM, McLaughlin H, Malka S, Samman PD. Mycosis fungoides--a disease of antigen persistence. *Br J Dermatol*. 1974;91(6):607-16.
  35. Edelson RL. Cutaneous T cell lymphoma: Mycosis fungoides, Se'zary syndrome and other variants. *J Am Acad Dermatol*. 1980; 2(2):89-106.
  36. Caporaso NE, Goldin LR, Anderson WF, Landgren O. Current Insight on Trends, Causes, and Mechanisms of Hodgkin's Lymphoma. *Cancer J*. 2009; 15(2):117-23.
  37. Lukes RJ, Craver LF, Hall TC, Rappaport H, Ruben P. Report of the nomenclature committee. *Cancer Res*. 1966; 26: 1311.
  38. Corthay A. How do Regulatory T Cells Work? *Scand J Immunol*. 2009; 70(4):326-36.
  39. Schmidt A, Oberle N, Krammer PH. Molecular mechanisms of Treg-mediated T cell suppression. *Front Immunol*. 2012;3: 51.
  40. Shevach EM. Mechanisms of Foxp3<sup>+</sup> T Regulatory Cell-Mediated Suppression. *Immunity*. 2009; 30(5):636-

- 45.
41. Förster R, Davalos-Miszlitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. *Nat Rev Immunol.* 2008; 8(5):362-71.
  42. Ochando JC, Yopp AC, Yang Y, Garin A, Li Y, Boros P, et al. Lymph node occupancy is required for the peripheral development of alloantigen-specific Foxp3<sup>+</sup> regulatory T cells. *J Immunol.* 2005; 174(11):6993-7005.
  43. Booth NJ, McQuaid AJ, Sobande T, Kissane S, Agius E, Jackson SE, et al. Different proliferative potential and migratory characteristics of human CD4<sup>+</sup> regulatory T cells that express either CD45RA or CD45RO. *J Immunol.* 2010; 184(8):4317-26.
  44. Seddiki N, Santner-Nanan B, Tangye SG, Alexander SI, Solomon M, Lee S, et al. Persistence of naive CD45RA<sup>+</sup> regulatory T cells in adult life. *Blood.* 2006;107(7):2830-8.
  45. Tomura M, Honda T, Tanizaki H, Otsuka A, Egawa G, Tokura Y. Activated regulatory T cells are the major T cell type emigrating from the skin during a cutaneous immune response in mice. *J Clin Invest.* 2010; 120(3):883-93.
  46. Ding Y, Xu J, Bromberg JS. Regulatory T cell migration during an immune response. *Trends Immunol.* 2012; 33(4):174-80.
  47. Liston A, Rudensky AY. Thymic development and peripheral homeostasis of regulatory T cells. *Curr Opin Immunol.* 2007; 19(2):176-85.
  48. Linehan DC, Goedegebuure PS. CD25<sup>+</sup>CD4<sup>+</sup> regulatory T-cells in cancer. *Immunol Res.* 2005; 32 (1-3):155-68.
  49. Sharma S, Yang SC, Zhu L, Reckamp K, Gardner B, Baratelli F, et al., Tumor cyclooxygenase-2/prostaglandin E2-dependent promotion of FOXP3 expression and CD4<sup>+</sup> CD25<sup>+</sup>T regulatory cell activities in lung cancer. *Cancer Res.* 2005; 65(12):5211-20.
  50. Baratelli F, Lin Y, Zhu L, Yang SC, Heuzé-Vourc'h N, Zeng G, et al. Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4<sup>+</sup> T cells. *J Immunol.* 2005; 175(3):1483-90.
  51. Baratelli F, Lin Y, Zhu L, Yang SC, Heuzé-Vourc'h N, Zeng G . et al. Prostaglandin E2 Induces FOXP3 Gene Expression and T Regulatory Cell Function in Human CD4<sup>+</sup> T Cells. *J Immunol.* 2005; 175(3):1483-90.
  52. Bodor J, Bopp T, Vaeth M, Klein M, Serfling E, Hünig T, et al. Cyclic AMP underpins suppression by regulatory T cells. *Eur J Immunol.* 2012; 42(6):1375-84.
  53. Choudhry MA, Uddin S, Sayeed MM. Prostaglandin E2 modulation of p59 fyn tyrosine kinase in T lymphocytes during sepsi. *J Immunol.* 1998; 160(2):929-35.
  54. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, et al. Regulatory CD4(+)/CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res.* 2001; 61(12):4766-72.
  55. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, et al. Conversion of peripheral CD4<sup>+</sup>CD25<sup>-</sup> naive T cells to CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med.* 2003; 198(12):1875-86.
  56. Zou W. Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol.* 2006;6(4):295-307.
  57. Maloy KJ, Powrie F. Regulatory T cells in the control of immunopathology. *Nat Immunol.* 2001; 2(9):816-22.
  58. Ai WZ, Hou JZ, Zeiser R, Czerwinski D, Negrin RS, Levy R. Follicular lymphoma B cells induce the conversion of conventional CD4<sup>+</sup> T cells to Tregulatory cells. *Int J Cancer.* 2009; 124(1):239-44.
  59. Kelley TW, Pohlman B, Elson P, Hsi ED. The ratio of FOXP3<sup>+</sup> regulatory T cells to granzyme B<sup>+</sup> cytotoxic T/NK cells predicts prognosis in classical Hodgkin lymphoma and is independent of bcl-2 and MAL expression. *Am J Clin Pathol.* 2007; 128(6):958-65.
  60. Tzankov A, Meier C, Hirschmann P, Went P, Pileri SA, Dirnhofer S. Correlation of high numbers of intratumoral FOXP3<sup>+</sup> regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma. *Haematologica.* 2008; 93(2):193-200.
  61. Mittal S, Marshall NA, Duncan L, Culligan DJ, Barker RN, Vickers MA. Local and systemic induction of CD4<sup>+</sup>CD25<sup>+</sup> regulatory T-cell population by non-Hodgkin lymphoma. *Blood.* 2008; 111(11):5359-70.
  62. Yang ZZ, Novak AJ, Stenson MJ, Witzig TE, Ansell SM. Intratumoral CD4<sup>+</sup>CD25<sup>+</sup> regulatory T-cell-mediated suppression of infiltrating CD4<sup>+</sup> T cells in B-cell non-Hodgkin lymphoma. *Blood.* 2006; 107(9):3639-46.
  63. Han Y, Wu J, Bi L, Xiong S, Gao S, Yin L, et al. Malignant B Cells Induce the Conversion of CD4<sup>+</sup>CD25<sup>-</sup> T Cells to Regulatory T Cells in B-Cell Non-Hodgkin Lymphoma. *PLoS One.* 2011; 6(12):e28649.
  64. Yamazaki T, Akiba H, Iwai H, Matsuda H, Aoki M, Tanno Y, et al., Expression of programmed death 1 ligands by murine T cells and APC. *J Immunol.* 2002; 169(10):5538-45.
  65. Berger CL, Hanlon D, Kanada D, Dhodapkar M, Lombillo V, Wang N, et al. The growth of cutaneous T-cell lymphoma is stimulated by immature dendritic cells. *Blood.* 2002; 99(8):2929-39.
  66. Rowden G, Phillips TM, Lewis MG, Wilkinson RD. Target role of Langerhans cells in mycosis fungoides:

- transmission and immuno-electron microscopic studies. *J Cutan Pathol.* 1979; 6(5):364-82.
67. Edelson RL. Cutaneous T-cell lymphoma: a model for selective immunotherapy. *Cancer J Sci Am.* 1998;4(2):62-71.
  68. Lutzner M, Edelson R, Schein P, Green I, Kirkpatrick C, Ahmed A. Cutaneous T-cell lymphomas: the Sezary syndrome, mycosis fungoides and related disorders. *Ann Intern Med.* 1975; 83(4):534-52.
  69. Dalloul A, Laroche L, Bagot M, Mossalayi MD, Fourcade C, Thacker DJ, et al., Interleukin-7 is a growth factor for Sezary lymphoma cells. *J Clin Invest.* 1992; 90(3):1054-60.
  70. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony stimulating factor plus interleukin 4 and down regulated by tumor necrosis factor- $\alpha$ . *J Exp Med.* 1994; 179(4):1109-18.
  71. Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M, et al., Immunologic tolerance maintained by CD25<sup>+</sup>CD4<sup>+</sup> regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev.* 2001; 182:18-32.
  72. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet.* 2001; 357(9255):539-45.
  73. Poppema S, van den Berg A. Interaction between host T cells and Reed-Sternberg cells in Hodgkin lymphomas. *Semin Cancer Biol.* 2000; 10(5):345-50.
  74. Marshall NA, Christie LE, Munro LR, Culligan DJ, Johnston PW, Barker RN, et al. Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood.* 2004; 103(5):1755-62.
  75. Atayar C, Poppema S, Blokzijl T, Harms G, Boot M, van den Berg A. Expression of the T-cell transcription factors, GATA-3 and T-bet, in the neoplastic cells of Hodgkin lymphomas. *Am J Pathol.* 2005; 166(1):127-34.
  76. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. , Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med.* 2004; 10(9):942-9.
  77. Lim HW, Hillsamer P, Banham AH, Kim CH .Cutting edge: direct suppression of B cells by CD4<sup>+</sup> CD25<sup>+</sup> regulatory T cells. *J Immunol.* 2005; 175(7):4180-3.
  78. Yoon NK, Maresh EL, Shen D, Elshimali Y, Apple S, Horvath S, et al. Higher levels of GATA3 predict better survival in women with breast cancer. *Hum Pathol.* 2010; 41(12):1794-801.
  79. Schreck S, Friebel D, Buettner M, Distel L, Grabenbauer G, Young LS, et al. Prognostic impact of tumour-infiltrating Th2 and regulatory T cells in classical Hodgkin lymphoma. *Hematol Oncol.* 2009; 27(1):31-9.
  80. Prabhala RH, Pelluru D, Fulciniti M, Prabhala HK, Nanjappa P, Song W ,et al. Elevated IL-17 produced by TH17 cells promotes myeloma cell growth and inhibits immune function in multiple myeloma. *Blood.* 2010; 115(26):5385-92.
  81. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature.* 2006; 441(7090):231-4.
  82. Chizzolini C, Chicheportiche R, Alvarez M, de Rham C, Roux-Lombard P, Ferrari-Lacraz S, et al. ,Prostaglandin E2 synergistically with interleukin-23 favors human Th17 expansion. *Blood.* 2008; 112(9):3696-703.
  83. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, et al. TGF- $\beta$ -induced Foxp3 inhibits TH17 cell differentiation by antagonizing ror $\gamma$ t function. *Nature.* 2008; 453(7192):236-40.
  84. León K, Pérez R, Lage A, Carneiro J. Modelling T-cell-mediated suppression dependent on interactions in multicellular conjugates. *J Theor Biol.* 2000; 207(2):231-54.
  85. Yu P, Gregg RK, Bell JJ, Ellis JS, Divekar R, Lee HH, et al. Specific T regulatory cells display broad suppressive functions against experimental allergic encephalomyelitis upon activation with cognate antigen. *J Immunol.* 2005; 174(11):6772-80.
  86. Tang Q, Adams JY, Tooley AJ, Bi M, Fife BT, Serra P, et al., Visualizing regulatory T cell control of autoimmune responses in nonobese diabetic mice. *Nat Immunol.* 2006; 7(1):83-92.
  87. Thornton AM, Shevach EM. CD4<sup>+</sup>CD25<sup>+</sup> immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med.* 1998; 188(2):287-96.
  88. Yu A, Zhu L, Altman NH, Malek TR. A low interleukin-2 receptor signaling threshold supports the development and homeostasis of T regulatory cells. *Immunity.* 2009; 30(2):204-17.
  89. Zhao DM, Thornton AM, DiPaolo RJ, Shevach EM. Activated CD4<sup>+</sup>CD25<sup>+</sup> regulatory T cells selectively kill B lymphocytes. *Blood.* 2006; 107(10):3925-32.