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The skewed balance between regulatory T cells and Th17 in chronic lymphocytic leukemia

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Running title: Imbalanced Treg and Th17 in CLL

Abstract

While regulatory T (Treg) cells maintain self tolerance and inhibit anti-tumor responses, T helper (Th)17 cells may enhance inflammatory and anti-tumor responses. The balance between these two important T cell subsets has been skewed in many immunopathologic conditions such as autoimmune and cancer diseases. B cell chronic lymphocytic leukemia (CLL) is the most common form of leukemia in Western Word and is characterized with monoclonal expansion of B lymphocytes. There is evidence which implies that the progression of CLL is associated with expansion of Treg and downregulation of Th17 cells. In this review, we will discuss about immunobiology of Treg and Th17 cells and their role in immunopathogenesis of CLL as well as their reciprocal changes during disease progression.

Keywords: chronic lymphocytic leukemia, regulatory T cells, Th17, inerleukin-17, balance

Introduction

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in the Western world [1], which is characterized by clonal expansion of $CD5^+$ B cells in periphery and lymphoid tissues [2]. The unmutated IG heavy chain variable (IGHV) region genes and higher expression of CD38 and ZAP-70 molecules are associated with poor prognosis in CLL patients [3].

CLL progression is mainly affected by the immune status of the CLL patients (Table 1). Several immune cells and mediators contribute to immunopathogenesis of CLL, such as regulatory T (Treg) cells and T helper (Th) 17 cells [4]. Increased absolute number of total T lymphocytes has been shown in CLL patients which was associated with expansion of $CD8^+$ T cells, result in a low CD4:CD8 ratio [5]. However, some phenotypic and functional abnormalities have been observed in these cells. While the expression of CD69, CD57 and HLA-DR was increased, the expression of CD28 and CD62L was decreased in these T cells. This phenotype may represent terminally differentiated effector memory T cells [6]. Functionally, it has been shown that both $CD4^+$ and $CD8^+$ T cells of CLL patients secrete higher amounts of IL-4 compared to normal subjects [7], which led to overexpression of Bcl-2 anti-apoptotic B CLL cells [8]. Interestingly, IL-4-producing $CD8^+$ T cells overexpress CD30 molecule which its ligation with CD30L on leukemic and normal B cells leads to proliferation and suppression of these cells, respectively [9]. The main effective anti-tumor $CD4^+$ T cell subset involved in the control of B cell malignancies are thought to be Th1 cells [10]. Contrasting results regarding the frequency and balance status between Th1/Th2 cells have been reported in CLL patients. While a shift from Th1 towards Th2 cells has been shown to be associated with CLL progression [11], decreased Th2 [12] or no change in Th1 [13] cell number have also been reported in CLL patients. However, it is suggested that Th cells enhance the expression of anti-apoptotic molecules including MCL1, BCL-XL, BFL1 and Survivin in leukemic B cells in CD40L dependent manner [14]. Moreover, different T cell secreted cytokines such as IL-2, TNF-α, IFNγ, IFN-α and IL-13 were also demonstrated to support leukemic cell proliferation and survival [15]. Importantly, gene expression profiling of purified T cells isolated from CLL patients showed modified expression of genes responsible for differentiation and cytoskeletal organization in $CD4^+$ T cells, and cytoskeletal formation, vesicle trafficking, and cytotoxicity in $CD8^+$ T cells [16].

Dendritic cells (DC) which are important modulators of T cell responses show some defects in their maturation and antigen presentation process in CLL patients [17]. Interestingly, it has been shown that leukemic cells induce impaired immunologic synapse formation between DCs and T cells [18].

Natural killer (NK) cells are one of the main immune cells in anti-tumor responses. Although it is reported that their frequency is increased in CLL patients [19], however, their cytotoxic function is markedly suppressed [20].

Little is known regarding the serum levels of different cytokines in CLL. Yan and colleagues have performed a comprehensive study in which the wide variety of cytokines divided in 3 cluster (CL) including CL1 (CXCL9, CXCL10, CXCL11, CCL3, CCL4, CCL19, IL-5, IL-12, and IFN-γ), CL2 (TNF-α, IL-6, IL-8, and GM-CSF), and CL3 (IL-1, IL-2, IL-4, IL-15, IL-17, and IFN-α) were investigated in CLL patients. They showed that patients with progressive disease had high CL1 and low CL2 or CL3 levels [21].

However, recent data indicates that the balance status between Treg and Th17 cells has an important role in progression or alleviation of various autoimmune and cancer diseases [22]. Treg and Th17 cells are two important subsets of T cells, which may be considered as main immunomodulators in different immunopathologic conditions, such as tumor. While Treg cells suppress the anti-tumor responses, Th17 cells may stimulate tumor rejection [22]. Recent data indicates that the CLL progression is associated with increased Treg and decreased Th17 numbers [23-25]. So, it may possible that the skewed balance between Treg and Th17 cells may assesses the disease outcome in CLL [22]. In this review, we tried to clarify the immunobiology of Treg and Th17 cells as well as their function in CLL progression. Moreover, we describe the factors responsible for their skewed balance which may be considered as worthy therapeutic targets in future.

Regulatory T cells

About a half century ago, Miller and colleagues were provided thymectomized mice and observed that mice were immunocompromized and developed autoimmunity and lymphoproliferative disease [26]. However, the term of immunosuppression was described in 1970s by Gershon *et al*. for the first time [27]. Subsequently, Sakaguchi *et al*. introduced the $CD4^+$ suppressor or regulatory T (Treg) cells with increased CD25 (IL-2 receptor α) expression [28]. Identification of Treg lineage-specific transcription factor, forkhead box transcription factor 3 (FoxP3), led to better discrimination of these cells from other T cell subsets [29]. It has been shown that development of Treg cells depends on T cell receptor (TCR) specificity, similar to conventional $CD4^+$ and $CD8^+$ T cells [30], but with very limited overlap in TCR repertoire [31]. During thymic development of thymocytes, variations in avidity and duration of TCR signaling are the key points in T cell differentiation [32].

There are different subsets of Treg cells in circulation [33]. Treg cells can derive from the thymus (tTregs), which are also known as natural Treg (nTreg). Some Treg cells can also arise extrathymically in the periphery (pTregs) following induction of Foxp3 in consequence of antigen exposure, which are also known as inducible Treg (iTreg) [34]. The differentiation of tTreg cells is mediated through recognition of self-antigen/MHC molecules expressed on thymic epithelial cells in the presence of costimulatory signals such as CD28 [35], but not transforming growth factor (TGF)-β [36]. However, maximum tTreg differentiation and its peripheral maintenance are depend on continuous TCR signaling in the presence of costimulatory signals, particularly IL-2 [37] and to lesser extent IL-7 and IL-15 [38]. It has been suggested that generation of pTreg occurs in response to foreign antigens such as food, allergens, and particularly commensal bacteria [39]. The suboptimal TCR signals and high concentration of TGF-β are pivotal for induction of pTreg cells [40]. Surprisingly, while CD28 signaling is crucial for tTreg development [35], it prevents pTreg induction [41], implying different costimulatory requirements for tTreg and pTreg cells.

Based on some newly identified markers such as Helios and Neuropilin-1 (Nrp-1) , it has been suggested that the majority of Treg cells in circulation are tTreg [42]. However, there is evidence which implies the expression of Helios and Nrp-1 in pTreg cells [43]. *In vivo* investigation of tTreg and pTreg TCR repertoire based on Nrp-1 expression showed that there is limited overlap between these two subsets of Treg cells which implies the different lineage development of tTregs and pTregs. Moreover, limited overlap was observed between Treg and conventional T cells which indicates pTreg cells constitute a very small subset of conventional T cells [43, 44].

Regulatory mechanisms of Treg cells can be divided into four mechanisms, including 1) secretion of inhibitory cytokines such as IL-10, TGF-β and IL-35, 2) direct cytolysis by granzymes, perforin, TRAIL-DR5 (TNF-related apoptosis inducing ligand-death receptor-5) and interaction with galectins, 3) metabolic disruption through IL-2 deprivation and the generation of adenosine, and 4) DC modulation [45].

Th17 cells

Th17 cell is characterized with the production of cytokines, such as IL-17A, IL-17F, IL-6, IL-9, IL-21, IL-22, IL-23, IL-26 and TNF-α, and enhances the clearance of fungi and extracellular bacteria and tissue inflammation in autoimmune diseases. It seems that there are three distinct subsets of Th17 cells (as shown in figure 1), including conventional Th17 cells which are known as Th17-β cells, Th17-23 and Th17-1 cells [46]. Th17-β cells produce IL-17A, IL-17F, IL-10, CCL20 and express CXCR6 chemokine receptor. The differentiation of these cells is induced through TGF-β in combination with IL-6, IL-1, and IL-21. Th17-23 cells produce higher levels of IL-22 and CCL9 in addition to IFN-γ, IL-17A and IL-17F and express CXCR3 chemokine receptor. The differentiation of Th17-23 cells is promoted by the combination of cytokines such as IL-6, IL-23, IL-1β, and IL-21 (in the absence of TGF-β) [46-49]. The third subset of Th17 cells is Th17-1 cell which is characterized with the production of IFN-γ and other Th17 common cytokines such as IL-17A, and expresses CCR6 and CXCR3 chemokine receptors. IL-1β, IL-21, IL-6, IL-12, TNF-α, and IL-23 are the main required cytokine microenvironment for development of Th17-1 cells [50, 51]. Among these cytokines, IL-12 can particularly induce IFNγ generation in Th17 which leads to induction of Th17-1 subset, that rapidly lose the IL-17 production ability, and shift toward Th1 cells [51]. These Th17-derived Th1 cells (known as non-classic Th1 cells) differ from conventional Th1 cells, because Th17-1 cells express genes such as RORC, CD161, CCR6, IL-4-induced gene 1 and IL-17 receptor E, which are absent in conventional Th1 cells [52]. Moreover, it has recently been shown that the transcription factors Runx1 or Runx3, in association with T-box transcription factor (T-bet), are essential for the generation of Th17-1 cells [53]. It is unknown whether Th17-1 cells represent a stable subtype of Th17 cells or a transitional phenotype between Th17 and Th1 cells [54]. However, it has been supposed that Th17-1 cells derive from Th17-23 cells in the presence of Th1-polarizing cytokine milieu, particularly IL-12 [55].

The retinoic acid-related orphan receptor (ROR)γt, RORα, and STAT3 are three main lineage-specific transcription factors that induce Th17 differentiation [56]. STAT3 induces RORγt and RORα under Th17-favoring conditions [57]. Other transcription factors such as RORγ [58], interferon regulatory factor (IRF)4 [59], B-cell-activating transcription factor (BATF) [60], RUNX1 [61], c-musculoaponeurotic fibrosarcoma (-Maf) [62], AHR [63], and hypoxia-inducible factor 1 (HIF1)α [64] are also involved in the differentiation and development of Th17 cells.

Contrary to above mentioned factors which promote Th17 development, there are several factors that inhibit Th17 differentiation. It has been shown that different hallmark cytokines or lineage-specific transcription factors for Th1, Th2 and Treg cells prevent commitment to Th17 lineage or inhibit the generation of Th17-derived cytokines [65]. IL-27 and interferon (IFN)β, are also the negative regulator of Th17 cells [66, 67]. The suppressor of cytokine signalling 3 (SOCS3) and Ets-1 transcription factor are other inhibitors of Th17 cells [68, 69].

Regulatory T cells in CLL

Beyer and colleagues showed the increased frequency of Treg cells in CLL patients, which was correlated with disease progression, for the first time in 2005 [23]. The latter reports were confirmed the data published by Beyer *et al*. [24, 70]. Moreover, Jack and coworkers showed that the increased frequency of Treg cells in CLL patients is due to increased formation through CD27-CD70 interaction and increased resistance to apoptosis via increased levels of Bcl-2 anti-apoptotic protein [71]. Following these findings, the increased frequency and absolute number of Treg cells in CLL patients and its association with disease progression were demonstrated, repeatedly [72-76] (as shown in table 2).

Although it seems that $CD4^+$ Treg cells are increased in CLL patients, little is known regarding the immunobiology of $CD8^+$ Treg cells in these patients. We have recently been shown that the frequencies of both $CD8^+FoxP3^+$ and $CD8^+CD25^+FoxP3^+$ Treg cells were increased in CLL patients and correlated with disease progression [4]. Other study has also been confirmed our experience [77]. Nunes and colleagues have also shown emergence of $CD8^+PD\text{-}1^+$ replicative senescent phenotype in early stage CLL and its correlation with disease progression [78].

The increased frequency of Treg cells not only was correlated with disease progression but also associated with different prognostic markers of CLL patients such as IGHV mutation status [72], CD38 expression [72], and the stage of disease [23, 24, 70, 73].

The functional capacity of Treg cells is another important issue in the immunobiology of Treg cells in CLL patients which is investigated in our [4] and some other studies [23, 77]. The first report regarding the Treg cells function in CLL was reported by Giannopoulos and coworkers which showed that the higher frequencies of Treg cells were correlated with decreased T cell responses against viral and tumor antigens [24]. Following this observation, Beyer *et al*. have been showed that the treatment of CLL patients with fludarabine led to decreased frequency and function of Treg cells as assessed by 5-bromo-2-deoxyuridine (BrdU) incorporation test [23]. Another group showed that the cytotoxicity of Treg cells was increased in CLL patients as investigated through the expression of CD107a marker on Treg cells. However, they observed any change in Fas-ligand expression between CLL patients and normal subjects. They have also been demonstrated that Treg cells kill isolated B cells in part through granzyme A. The suppressive function of Treg cells on the proliferation of allogeneic peripheral blood mononuclear cells (PBMCs) was also intact as analyzed by Alamar Blue proliferation assay [79]. The intact suppressive function of Treg cells in CLL patients has also been reported by other studies as detected via CFSE (carboxyfluorescein diacetate succinimidyl ester) proliferation assay [72, 77]. It is suggested that Treg cells in CLL patients deprive effector T cells from IL-2 through secretion of soluble CD25, so which prevent anti-tumor responses exerted by effector T cells [80]. Recently, we have also been showed that the soluble factors in the supernatant of *in vitro*-stimulated Treg cells could inhibit the proliferation of both effector T cells and B cells from CLL patients similar to normal subjects [4]. Most recently, Rissiek *et al.* have been studied the composition and function of circulating T cells during the stages of monoclonal B cell lymphocytosis (MBL), early, and more advanced CLL. They showed that suppressive Treg cells arise early, during the pre-CLL stage of MBL. Moreover, T cell functional assays demonstrated an increasingly suppressive regulatory function initiating at the MBL stage. An increasingly suppressive phenotype has been observed during disease progression, which can be in part reversed by chemoimmunotherapy [81].

It should be clarified that the overall outcome resulted following the inhibition of Treg cells in CLL disease will be useful or not. Recently, it has been demonstrated that the treatment of CLL patients with thalidomide leads to better prognosis and reduced Treg cells [82]. Moreover, these reduced Treg cells were mainly Nrp-1^{+} which are tTreg [83]. Furthermore, vaccination of CLL patients with tumor lysate loaded-dendritic cells was associated with reduced Treg cells [84]. In addition, our recent data showed that expansion of Treg cells in association with disease progression in CLL patients could reduce NKT-like cells which have anti-tumor potential [85, 86]. Thus, it seems that downregulation of Treg cells will associated with better prognosis in CLL patients, however, these assumptions cannot be justified without performing comprehensive studies for clarifying of precise details regarding Treg cells in immunopathogenesis of CLL.

Th17 cells in CLL

Little is known regarding the immunobiology of IL-17 producing T cells in pathogenesis of cancer. There are some studies about Th17 cells in various solid tumors and a few haematological malignancies [87]. The protective role of Th17 cells has been implied in different solid tumors [88]. In contrast to solid tumors, little is known about the immunobiology of Th17 cells in hematologic malignancies, particularly CLL [89]. Initial studies about the IL-17 producing T cells in CLL were recently performed in a limited sample size and showed highly variable results [90-92]. Following these observations, we showed that the frequencies of both Th17 and Tc17 cells were markedly decreased in CLL patients and correlated with disease progression. Moreover, we found that reduced frequency of Th17 cells was associated with unmutated IGHV, which implied the protective role of Th17 cells in CLL. However we observed any association between the number of IL-17 producing T cells and other prognosis markers such as CD38 or ZAP-70 expression [93]. It has recently been demonstrated that the frequency of IL-17 producing follicular Th cells was increased in CLL patients and correlated with advanced CLL stages [94]. It should be noted that this study cannot imply the deleterious role of Th17 or Tc17 cells, because fTh cells can secrete different mediators in comparison with Th17 or Tc17 (such as IL-21) which may describe fTh-mediated tumor promotion [95]. Consistent with our results, Idler and colleagues have reported that treatment of CLL patients with lenalidomide led to induction of Th17 cells, which implies the protective role of Th17 cells in CLL [92]. Increased frequency of IL-17 producing T cells has also been reported in CLL/SLL patients compared to other B cell malignancies [90]. Another group has also been reported that increased frequency of Th17 cells was correlated with better prognostic markers and longer survival. In addition, non-Th17 IL-17A-expressing cells were present in CLL spleens as maturing granulocytes and mature mast cells, suggesting the microenvironmental milieu in leukemic spleens enhances the recruitment and expansion of Th17 and other IL-17-expressing cells [96]. Hus and coworkers have also suggested the protective role for Th17 cells in CLL patients. They showed that the frequency of Th17 cells and IL-17A levels were significantly decreased in advanced disease stages. Furthermore, the frequency of Th17 cells was lower in patients who died or responded to first-line therapy with fludarabine compared to surviving patients and non-responders, respectively. IL-17A inversely correlated with the time from CLL diagnosis to the start of therapy and was lower in patients who required treatment during follow-up. Moreover, Th-17 and IL-17A values were adversely correlated with bad prognostic factors such as 17p and 11q deletion, CD38 and ZAP-70 expression. The results of this study suggest that Th17 may play a beneficial role in CLL [97]. Altogether, it seems that the frequency of Th17 cells trends to be decreased with CLL progression implying the protective role of these cells in CLL patients (Table 3).

Although some data regarding the protective mechanisms exerted by Tc17 cells have already been reported [98], little is known regarding the protective mechanisms of IL-17 producing cells for the control of tumor cells. However, considering pro-inflammatory function of these cells, it seems that they provide an inflammatory microenvironment in which tumoral cells could be killed. Some subsets of Th17 cells have been shown to secret both IFNγ and IL-17 cytokines which are effective factors in anti-tumor responses [99]. It is suggested that Th17-β cells suppress anti-tumor responses in part through secretion of IL-10 and generation of adenosine via expression of CD73 and CD39. Controversially, it is supposed that Th17-23 cells enhance anti-tumor responses in part through secretion of IFNγ and degeneration of adenosine to inosine via adenosine deaminase expressed in these cells [55]. It doesn't seem that Th17 cells use contact-dependent mechanisms in their anti-tumor responses. However, there is no sufficient data regarding the functionality and precise mechanisms by which Th17 cells exert their protective role in CLL immunopathogenesis. Investigation of IFNγ-producing Th17 cells in CLL patients might be critical for better understanding the role of these cells in immunopathogenesis of CLL.

The skewed balance between Treg and Th17 in CLL

Generally, it is accepted that the progression of autoimmune diseases is associated with downregulation of Treg and expansion of Th17 cells [22, 100]. On the other hand, cancer progression is supposed to be linked to Treg expansion and Th17 reduction [93]. The skewed balance between these cells may result in the burden of tissue inflammation, autoimmune diseases or cancer [46]. Although several groups have been investigated this issue in autoimmune diseases, little is known regarding their imbalance in cancer [101]. In order to study this balance in CLL, we have recently analyzed different subsets of Treg and IL-17 producing T cells in these patients. Our results showed that the increased frequencies of different Treg subsets are associated with a downregulation of Th17 and Tc17 cells. Moreover, the imbalance between mRNA levels of lineage specific transcription factors FoxP3 and RORγt were confirmed the imbalance between Treg and IL-17 producing T cells [93]. Consistently, another group was recently demonstrated that the frequency of Th17 cells positively correlated with iNKT and adversely with Treg cells which implied the beneficial role of Th17 cells in CLL [97]. It has also been demonstrated that some therapeutic drugs such as lenalidomide trends to induce Th17 and inhibits Treg cells which was associated with the better CLL prognosis [92]. Imbalanced frequency of Treg/Th17 in CLL patients is also reported by other investigators [75, 102]. Recently, it has been suggested that $CD39^+$ Treg cells can suppress Th17 cells [103]. Moreover, it has recently been showed that $CD39^+$ Treg cells inhibited development of Th17 cells in human [104] and animal cancer models [105]. So which, we have enumerated $CD39^+$ Treg cells in CLL patients. Our data showed that expansion of $CD39^+$ Treg cells was associated with downregulation of IL-17 producing T cells (Th17 and Tc17). However, there was no significant correlation between the expansion of $CD39^+$ Treg and downregulation of IL-17 producing T cells. Thus, it may be possible that these two subsets of T cells are modulated differently in CLL [93]. Consistently, Pulte and coworkers found that the frequencies of both $CD4^+CD39^+$ and $CD8^+CD39^+$ T cells were increased in CLL patients and correlated with disease progression [106]. It should be noted that their investigated populations were not Treg and solely represented CD39 molecule expressing T cells. In addition to Treg cells, there is evidence which implies malignant B cells as potent modulator of balance status between Treg and Th17 cells. It has been shown that malignant B cells inhibit IL-17 producing T cells and stimulate Treg development in non-Hodgkin's lymphoma by mechanisms in which CD27-CD70 or CD28-B7.1,2 interactions are involved [90]. Although the precise mechanism(s) of this modulation is(are) not clarified, it may be possible that malignant B cells secrete high amounts of TGF-β [107] and to lesser extent IL-2 [79] which their combinatorial effect is critical stimulator of Treg induction and Th17 inhibition [22]. Furthermore, since Th17 cells express IL-10R [108] and leukemic B cells are one of the main sources of IL-10, it may possible that leukemic B cells suppress Th17 cells in IL-10-dependent manner [109]. On the other hand, IL-10 produced by leukemic B cells not only can suppress Th17 cells, but also may induce Treg cells. Different modulators of Th17 differentiation including cytokines (such as IL-25 and IL-27) [46] may be involved in observed imbalance during disease progression which are not studied in CLL until now. The nurse-like cells and hepatocyte growth factor were recently identified as Treg inducer factors in CLL patients. It is demonstrated that hepatocyte growth factor is in large amounts in serum of CLL patients and interacts with its receptor on leukemic B cells, nurse-like cells and monocytes, known as c-MET, which leads to increased leukemic B cells survival. The mechanism by which nurse-like cells and monocytes induce Treg and inhibit other T cell subsets is in part through production of TGF-β, IL-10 and indoleamine 2,3-dioxygenase enzyme [110]. Thus, it may possible that during disease progression, nurse-like cells induces Treg and inhibits Th17 cells. A vascular growth factor (VEGF) receptor, Nrp-1, on Treg cells is another inducer of Treg cells in CLL patients. Recently, Piechnik and colleagues reported that the expression of Nrp-1 was significantly higher on leukemic lymphocytes, Treg and plasmacytoid DCs of CLL patients compared to normal individuals. The positive correlation was also detected between expression of VEGF receptors (FLT1, NRP1) and FoxP3 expression. Moreover, treatment with thalidomide which was previously showed decreased Treg cells, reduced the expression of Nrp-1 on Treg cells *in vitro* [83]. Thus, it seems that tTreg cells that express Nrp-1 may potently stimulated by VEGF during CLL progression. Beside the above mentioned factors that have identified as modulator of Treg/Th17 balance in CLL (Table 4), several other factors can also control their balance that are elusive in CLL (as shown in figure 2).

The mTOR is an important factor that can regulate Treg/Th17 balance in many immunopathologic conditions [111]. While the suboptimal activation of mTOR induces Treg, potent activation of mTOR, particularly mTORC1, induces Th1 and Th17 phenotypes [112]. However, treatment of CLL patients with different mTOR inhibitors in order to arrest the cell cycle was not successful, which might be in part due to its stimulatory function on Treg cells which was not addressed in these trials [113].

The transcription factor hypoxia inducible factor-1 (HIF-1) which is expressed in nearly all mammalian cells is another important factor that can regulate Treg/Th17 balance [114]. It has been demonstrated that HIF-1 can stimulate differentiation, expansion, function and survival of Th17 cells both *in vitro* and *in vivo* through direct and indirect manner. On the other hand, HIF-1 downregulates the development of $FoxP3^+$ Treg cells [64]. However, as HIF-1 is overexpressed in leukemic CLL B cells and is associated with disease progression [115], it seems that HIF-1 cannot be considered as worthy tool for downregulation of Treg and expansion of Th17 cells.

Lipid oxidation can skew the Treg/Th17 balance toward Treg cells. Treg cells are more dependent to energy resulted from lipid oxidation (and not glycolytic process) than other T cell subsets [116]. On the other hand, lipid oxidation inhibits the development of Th17 cells, *in vitro* [116]. It has been reported that lipid oxidation is a mechanism of resistance to glucocorticoids -mediated cytotoxicity in CLL, and peroxisome proliferator-activated receptor (PPAR)α inhibition is an approach to improve the therapeutic efficacy of glucocorticoids [117]. Thus, inhibition of lipid oxidation not only facilitates glucocorticoids-mediated cytotoxicity, but also prevents Treg expansion.

AMP-activated protein kinase (AMPK), which stimulates lipid metabolism and inhibits mTOR, is another Th17/Treg axis modulator [118]. It has been demonstrated that both tTreg and pTreg showed increased AMPK activity [116]. Moreover, AMPK is a potent negative regulator of mTORC1 [111]. Thus, AMPK activity drives Treg/Th17 balance toward Treg cells, however, as AMPK activation is involved in apoptosis of leukemic B cell [119], its inhibition may lead to tumor expansion in CLL.

PPARs are recently known as Treg/Th17 balance modulators. PPARs are transcription factors which are activated by fatty acids and seen as different isoforms including α, β/δ, and γ, each with different ligand [120]. It has been shown that PPARγ activation inhibits the induction of RORγt, STAT3 and IL-17, so which it inhibits the differentiation of Th17 cells [121]. In addition, activation of PPARβ/δ suppresses both Th17 and Th1 cells and increases IL-10 production [122]. Moreover, ligation of PPARα shifts Th1 response toward Th2 and declines the generation of pro-inflammatory cytokines [120]. On the other hand, ligation of PPARγ and to lesser extent PPARα not only decreased Th17 responses, but also induced Treg development [123, 124]. Interestingly, while mTOR activates PPARγ, it inhibits PPARα [111], which implies very complex network in regulating Treg/Th17 balance. Current data shows that while inhibition of PPARα attenuates CLL progression, PPARγ ligation leads to apoptosis of leukemic B cell [117, 125]. So which it seems that PPARα may be better target for reversing Treg/Th17 imbalance in CLL patients compared to PPARγ.

The cytosolic fatty acid-binding proteins (FABPs) are other players in skewed balance between Treg/Th17. It has been shown that FABPs could promote Th17 development in part through downregulation of PPARγ [126].

Liver X receptors (LXR) which have two different isoforms including LXRα and LXRβ, express in Th cells and regulates gene products involved in metabolism of cholesterol and fatty acid [127]. It has been shown that the ligation of LXR inhibited expression of RORγt, IL-17 and Th17-related molecules [128]. This suppression was in part through induction of sterol regulatory element binding protein (SREBP-1), which binds to and suppresses AHR [129]. Although there is no data regarding the effect of LXR on Treg cells in CLL, however, its ligation has been led to apoptosis in leukemic B cells from CLL patients [130].

The hormone nuclear receptor, estrogen-related receptor (ERR)α is another candidate in regulating Treg/Th17 balance. It is suggested that ERRα ligation activates Th17 development, while inhibits Treg induction [131]. Moreover, in pre-clinical study, it has been shown that ligation of estrogen-related receptor was associated with ameliorative effects [132].

The relative amino acid abundance leads to activation of mTORC1 which in turn stimulates Th17 induction and inhibits Treg development [133]. On the other hand, amino acid starvation leads to downregulation of IL-17 producing T cells [134].

It has been shown that the activation of PI3K/Akt pathway leads to development of Th17 cells in part through stimulation of mTORC1, accumulation of RORγt and inhibition of Th17 inhibitor, Gfi-1 [135]. Since PI3K/Akt is regulator of both mTOR and HIF-1, it can be as an important modulator of Treg/Th17 axis [136]. Moreover, factors that interfere with PI3K/Akt pathway such as programmed death-1 (PD-1) inhibit Th17 differentiation and induce Treg phenotype [137]. However, as the stimulation of PI3K/Akt pathway stimulates the proliferative capacity of leukemic cells [138], it seems that blocking this pathway in order to expansion of Th17 cells may be rational. However, inhibition of this pathway may induce apoptosis in B-CLL cells [139].

The vitamin A metabolite, RA [140] and vitamin D are other factors, which can skew the Treg/Th17 balance toward anti-inflammatory status via suppression of Th17 development and enhancing Treg differentiation [141]. Although there is controversial results regarding the apoptotic effects of IL-21 on leukemic B cells [95], it seems that vitamin D insufficiency is associated with CLL poor prognosis [142].

The inflammatory lipid mediators may also play an important role in modulation of Treg/Th17 balance. It is reported that leukotriene (LT) B4 and prostaglandin (PG) E2 reduced the Treg frequency and Foxp3 expression in dose-dependent manner. On the other hand, LTB4 increases and PG2 decreases the IL-17 production and RORγt expression [143]. Available data indicates that increased levels of LTB4 [144] and PGE2 [145] is associated with CLL progression, so which targeting lipooxygenase or cyclooxygenase pathways may be useful in correction of Treg/Th17 imbalance in CLL patients.

The Treg/Th17 interaction can also regulate their balance. Fletcher and colleagues demonstrated that the CD39 expressing Treg cells could attenuate IL-17 generation in multiple sclerosis [103]. CD39 in cooperation with CD73 can cleave adenosine triphosphate (ATP) to immunosuppressive adenosine [103]. Thus, a decreased frequency of $CD39^{+}$ Treg cells can lead to development of Th17 cells as we showed in CLL patients [93]. However, this hypothesis needs to investigation of adenosine receptor expression in Th17 cells.

The phenotype conversion or T cell plasticity may be another factor responsible for Treg/Th17 imbalance. It has been observed that Treg cells can express Th17-related transcription factors RORγt and RORα and produce IL-17 [146]. Moreover, FoxP3 binds to and inhibits both RORγt and RORα in dose dependent manner [147]. It has been demonstrated that STAT3 induces HIF-1 which binds to and targets Foxp3 for proteosomal degradation [64]. Furthermore, FoxP3 binds to and inhibits Runx transcription factor which is positive regulator of Th17 differentiation [61]. These results imply the role of pro-inflammatory or immunosuppressive microenvironment in phenotype conversion of Treg or Th17 cells. Moreover, it is proposed that epigenetic modification can also enhance the Treg conversion into Th17 cell [148].

Since TGF-β can induce both FoxP3 and RORγt in different cytokine milieu in a Smad2-/Smad3-dependent manner [147], it seems that TGF-β plays a central role in T cell fate decision.

Conclusion and future perspective

As discussed above, the progression of CLL was associated with upregulation of Treg and downregulation of IL-17 producing T cells [93]. This finding implied that Treg cells could be as tumor promoting cells whereas Th17 might be protective during CLL progression. However, the relevance of increased frequency of Treg and decreased Th17 cells to the expansion of leukemic B cells and disease progression are not fully understood. As Treg cells could suppress effector T cell subsets [4, 23], so which they might negatively influence the control of leukemic B cells expansion. Moreover, the mechanism(s) by which Th17 cells can exert their anti-tumor effects in CLL is elusive. It may be possible that they suppress tumor progression through production of IFN-γ [55].

On the other hand, treatment of CLL patients with some therapeutic agents such as lenalidomide was associated with promotion of Th17 and inhibition of Treg cells and the better CLL prognosis [92]. The immunomodulatory effects of lenalidomide have also been investigated in other immune cells. It has been shown that lenalidomide can enhance anti-tumor function of NK, NKT-like and $CD4^+$ T cells [149]. However, little is known regarding the immunomodulatory effects of several novel therapeutic agents such as ibrutinib, idelalisib, or ABT-199 [150]. In spite of these data, there are many unanswered questions regarding the immunobiology of different subsets of Treg and Th17 cells in CLL which require further investigations. However, it seems that the control of CLL needs to inhibition of Treg expansion and induction of Th17, concomitantly. Consistently, there are several factors (as discussed in previous section) which could affect the Treg/Th17 balance. So, combinatorial regulation of such Treg/Th17 modulators might be as a promising approach in treatment of CLL. In addition, identifying the new factors which can regulate Treg/Th17 balance might be as interesting field for investigators.

Conflict of interest statement

None of the authors has any conflict of interest to declare.

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None.

Executive summary
Background
• CLL is the most common leukemia in the Western Word which is incurable until now.
• The imbalanced Treg/Th17 frequency has been observed in several autoimmune and cancer disease.
• The balance status between Treg and Th17 cells may influences immunopathogenesis of CLL.
Regulatory T cells
• Treg cells generally divides into two groups, known as thymus-derived tTreg and peripherally induced pTreg cells.
• FoxP3 is the lineage-specific transcription factor and is indispensable for Treg development.
• Treg cells maintain self tolerance and suppress anti-tumor responses.
Th17 cells
• Th17 cells typically divided into three groups, known as Th17-β, Th17-23 and Th17-1 cells.
• RORγt and STAT3 transcription factors are the main inducers of Th17 differentiation.
• It seems that Th17 cells inhibit tumor progression.
Treg cells in CLL
• The frequencies of different subsets of Treg cells are increased in CLL patients and correlated with disease progression.
• The function of Treg cells is intact in CLL patients.
Th17 cells in CLL
• Th17 cells are decreased in CLL patients.
• It seems that Th17 cells are protective during disease progression.
The skewed Treg/Th17 in CLL
• Downregulation of Th17 cells is associated with Treg expansion during CLL progression.
Conclusion & future perspective
• CLL progression is associated with Treg expansion and Th17 reduction.
• Several factors can affect Treg/Th17 balance which are elusive in CLL.
• There is no comprehensive study regarding the different Th17 subsets in CLL.
• The precise anti-tumor mechanisms exerted by Th17 cells are unknown.

Figure legends

Figure 1: The differentiation of different subsets of Th17 cells. There are three subsets of Th17 cells including Th17-β, Th17-23 and Th17-1. While differentiation of Th17-β cells depends on TGF-β, differentiation of Th17-23 and Th17-1 is TGF-β independent. Non-classic Th1 cells derive from Th17-1 cells and differ from conventional Th1 cells.

Figure 2: Balance status between Treg and Th17 cells in cancer. The factors that influence the development of Treg and Th17 cells are shown. While the development of Th17 cells inhibits tumor progression, Treg expansion can promote tumor burden. Treg: regulatory T cell, IL: interleukin, Th: T helper, FoxP3: forkhead box protein P3, TGF-β: transforming growth factor-β, RA: retinoic acid, LTB4: leukotriene B4, PGE2: prostaglandin E2, oxLDL: oxidized-low density lipoprotein, mTOR: mammalian target of rapamycin, HIF-1: hypoxia inducible factor-1, AMPK: AMP-activated protein kinase, PPAR: peroxisome proliferator-activated receptor, FABPs: fatty acid-binding proteins, LXR: Liver X receptor, ERR: estrogen-related receptor, Nrp-1: neuropilin-1, HGF: hepatocyte growth factor

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