Immunological aspects of adult T-cell leukemia/lymphoma (ATLL), a possible neoplasm of regulatory T-cells

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Abstract: Adult T-cell leukemia/lymphoma (ATLL) is a distinct disease caused by the first discovered human oncogenic retrovirus, human T-cell leukemia virus type-1 (HTLV-1). The peculiarity of this disease is not only in its causative agent HTLV-1 but also in the character of leukemia cells. ATLL cells express the mature helper/inducer T-cell antigens, CD2, CD3, CD4 and CD5 but usually lacking CD8. Despite of CD4 expression, it has long been known that ATLL cells exhibit strong immunosuppressive activity in vitro. Notably, ATLL patients are in severely immunosuppressed conditions and this causes higher incidences of opportunistic infections than other types of leukemia and lymphoma. Since ATLL cells constitutively express CD25, this prompted investigators to study ATLL cells from the viewpoint of regulatory T cells (Treg cells). ATLL cells satisfy all the criteria of Treg cells, as they express Foxp3, the master gene of Treg lineage, the glucocorticoid-induced TNF receptor (GITR), and the cytotoxic T-lymphocyte associated molecul-4 (CTLA-4). Moreover, other profiles including chemokine receptor expression also support that ATLL is a neoplasm of Treg cell origin. Here we review the immunological aspects of ATLL cells and discuss this cell origin.

Introduction

ATLL was proposed as a new disease entity in 1977 because of its limited distribution of the patients' birthplaces in the Kyushu area of Japan and more suggestively its peculiar clinical and laboratory findings [1]. Hypercalcemia and involvement of the skin and visceral organs, especially that of the lung, liver, and gastro-intestinal tracts, are frequent signs of the disease, and ATLL cells show a characteristic morphology with multi-lobulated nuclei called flower cells (Fig. 1). In 1981, its causative agent HTLV-1 was discovered [2-3], and the geographic clustering of the HTLV-1 carriers and HTLV-1-related diseases was recognized: Japanese in Asia, Melanesians in near Oceania, blacks in central Africa and the Caribbean basin, and American Indians in Central and South America [4]. The regional clustering is explained by mother-to-child transmission of the virus by breast-feeding in closed communities [5,6]. Although ATLL is an all encompassing term that is designed to highlight monoclonal nature of the disease and define it as a condition that involves the monoclonal proliferation of HTLV-1-infected T-lymphocytes (Fig. 2), its clinical behavior is quite diverse among patients. ATLL is thus subclassified into four subtypes; the smoldering and chronic types are indolent subtypes and the

acute and lymphoma types are aggressive subtypes [7]. Patients with the indolent subtypes survive several years even without chemotherapy, but the prognosis of the aggressive subtypes is extremely poor and their median survival time is only 13 months even in the recent multicenter clinical trial performed in Japan [8].

One of the causes of poor prognosis comes from suppression of their immunosurveillance system, and the patients with aggressive subtypes are sometimes found with opportunistic infections such as *Pneumocystis jiroveci* pneumonia, fungal infection, or *Cytomegalovirus* infection before and/or during treatment (Table 1) [9], which makes it difficult to start or continue chemotherapy. Moreover, some patients show conditions similar to hyper-IgE syndrome that is characterized by recurrent staphylococcal infections of the skin, chronic eczema-like rashes, eosinophilia, and markedly elevated serum IgE levels [10,11]. Another piece of evidence for the immunosuppression that occurs in ATLL is the low response to the tuberculin skin test that is used to assess the general status of individual cellular immunity in Japan. Since the BCG vaccination is given to all individuals who show a negative reaction to the test, 70 to 80% of Japanese around 60 years of age, the median age of ATLL patients, show a positive

reaction.　The prevalence of positivity for the tuberculin skin test in acute type and lymphoma type ATLL patients is very low (15% and 25%, respectively), which is significantly different from its prevalence in other lymphoproliferative disorders (p<0.05) [12].　Notably, tuberculin skin test-negative patients produce a positive reaction after they have achieved complete remission [12], suggesting that ATLL cells play an immunosuppressive activity *in vivo*.

The immunological characteristics of ATLL cells

　　1. T cell-related markers (Table 2)

　　ATLL cells display an activated mature helper/inducer T-cell phenotype: CD1-, CD2+, CD3+, CD4+, CD5+, CD7-, CD8-, and HLA-DR+ (Fig. 3) [13]. T-cell receptors (TCR) are always α/β heterodimers and no patients with γ/δ heterodimers have been reported.　The density of cell surface CD3/TCR molecules is usually low compared with normal T cells or other mature T-cell malignancies despite their high mRNA expression, and this discordance is also believed to be one of the characteristics of ATLL cells [14, 15]. Although more than 80% cases show the CD4+CD8- phenotype, some cases show a double positive (CD4+CD8+), double negative (CD4-CD8-), or even

CD8+ single positive phenotype [16, 17]. Loss of CD2 and/or CD5 antigens is also not rare, and the prognosis of these aberrant cases is usually poor [16]. Moreover, ATLL cells sometimes change surface phenotype to aberrant forms, gain CD8 or lose CD4, at relapse or at the exacerbation phase [18, 19].

Mature T cells are divided into two categories according to their antigen stimulation, naïve T cells before stimulation and memory T cells after stimulation, which are distinguished by the two isoforms of leukocyte common antigen family (LCA, CD45); naïve T cells are CD45RA+ and memory T cells are CD45RO+. ATLL cells are usually CD45RA-CD45RO+ with a few exceptional cases being CD45RA+CD45RO+ double positive [19]. Consistent CD45RO expression interpreted as indicating that the target cells for HTLV-I-induced neoplastic transformation are helper memory T cells. Alternatively, this may simply reflect the activated T cell nature of ATLL cells because it has been reported that CD45RO expression is induced following T-cell activation [20].

ATLL cells express CD30 [21, 22], a member of the TNF receptor superfamily known to be expressed on activated T and B cells, and this result also supports the idea that ATLL cells are in an activated phase. Elevated CD30 expression is considered one of the causes of the constitutive

NF-κB activation observed in ATLL cells, which is believed to be involved in ATLL oncogenesis [22, 23]. Another T cell activation marker, CD44, is also expressed in ATLL cells [24]. In contrast, although CD26/dipeptidyl peptidase IV is also known to be upregulated during T cell activation, ATLL cells either lack it or profoundly down-regulate its expression [25, 26]. The pathological significance of this down-regulation has not been elucidated.

2. Cytokines and cytokine receptors (Table 2)

Helper T cells are divided into two subsets, T helper 1 (Th1) and T helper 2 (Th2), according to their cytokine production patterns [27]. Th1 cells produce interleukin 2 (IL-2), interferon γ (IFN-γ), and tumor necrosis factor β (TNF-β) and are involved in cell-mediated inflammatory reactions, *i.e.* Th1 cells induce delayed-type hypersensitivity (DTH). In contrast, Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 and are involved in humoral immune responses, *i.e.* Th2 cells help antibody production, particularly IgE responses. The production of IL-2, IL-6, IL-10, and IL-13 is, however, not tightly restricted between the two subsets in humans as is the case in murine T cells, moreover, both subsets produce IL-3, TNF-α, and granulocyte-macrophage colony-stimulating factor (GM-CSF) [27]. There are other two subsets, Th0 and Th3; Th0 produce both Th1 and Th2 cell

cytokines, and Th3 produces IL-10 and transforming growth factor-β (TGF-β).

These overlapping characteristics make it difficult to determine which

objective T cells belong to which subset. In a practical and simplified

setting, it may be possible to say that Th1 cells are characterized by

producing IFN-γ and that Th2 cells are characterized by producing IL-4.

Since ATLL cells show a characteristic cell surface phenotype, ATLL is

believed to be derived from a distinct helper T cell subset, and hence ATLL

cells may show a restricted cytokine production pattern. In the analysis of

ATLL cell lines as pure ATLL cell samples, ATLL cell lines from different

patients indeed showed the same cytokine producing profile, namely, they

produced IFN-γ, IL-6, IL-8, LD78/MIP-1α, TNF-α, M-CSF, and GM-CSF but

did not produce IL-1α, IL-1β, IL-2, IL-4, IFN-α, or G-CSF [28, 29]. The

positive production of IFN-γ and the negative production of IL-4 suggest that

ATLL cells are of Th1 subset origin. This interpretation, however, faces the

difficulty that HTLV-1 Tax protein influences for cellular genes including

those cytokines. HTLV-I possesses a unique 3' region in its genome,

designated as pX, which encodes the viral transactivator Tax protein. Tax

transactivates not only its own gene expression but also many kind cellular

genes such as IL-1α, -1β, -2, -3, -4, -5, -6, -8, -10, -15, GM-CSF, G-CSF, TNF-α,

TNF-β, IFN-γ, LD78/MIP-1α, MIP-1β and MCP-1, PDGF, TGF-β, NGF, and

PTHrP [30, 31].   Accordingly, the unexpected production of IL-6 in ATLL

cells may be attributable to the Tax protein, or alternatively, the

Th1-Th2-dichotomy may not be fitting to determine neoplastic cell origins.

Th17, another newly described lineage of CD4 T cells, is characterized by the

production of IL-17, IL-17F, and IL-6 [32].   Although it has been

demonstrated that the skewing of helper T cells toward either Th17 cells or

Treg cells is mutually exclusive in murine systems [32, 33], there is no such

evidence in humans [34].   It has been reported that Tax protein induces

IL-17 gene expression in T cells [35], but IL-17 production in primary ATLL

cells has not yet been examined.

In contrast to such ambiguous cytokine production, ATLL cells show a

distinct cytokine receptor expression profile.   One of the most characteristic

features is the expression of the IL-2 receptor (IL-2R) αchain (CD25) [36].

IL-2R consists of three components, the α-chain, β-chain, and γ-chain.

IL-2R γ-chain is called common γ (γc), and the receptors that share γc are

called the γc-receptor family; IL-4R, IL-7R, IL-9R, IL-15R, and IL-21R belong

to this family.   Interestingly, ATLL cells express all these members except

IL-9R, and more importantly, these receptors are functional and IL-2, IL-4,

IL-7, IL-15, and IL-21 stimulate the proliferation of primary ATLL cells [36-39], suggesting their pathological roles.   Among them, IL-15 is unique in the sense that its mRNA and/or protein are detected in a variety of human cells and tissues, such as monocytes, macrophages, dendritic cells, the skin, the liver, the kidneys, and the lungs etc, although other cytokines are entirely produced from T cells in the immune system.   The frequent invasion and rapid proliferation of ATLL cells in a variety of tissues and organs may thus be attributable to local IL-15 production [40].   HTLV-1 Tax protein transactivates the genes for IL-2Rα and IL-15Rα [31, 41, 42], whose function may be related to the deregulated expression of these receptors in ATLL.

3. Chemokines and chemokine receptors (Table 2)

Chemokines induce the directed migration of target cells by interaction with chemokine receptors that have seven transmembrane domains coupled to a G-protein [43-45].   The differential expression of chemokine receptors on lymphocytes correlates with their tissue-specific homing and immune functions.   It has been reported that Th1 and Th2 cells differentially express chemokine receptors; Th1 cells preferentially express CXCR3 and CCR5, whereas Th2 cells express CCR4, and to a lesser extent, CCR3 [46].

Since primary ATLL cells express CCR1, CCR4, CCR7, CCR8, CCR10, and CXCR4 but hardly express CCR2, CCR3, CCR5, CCR6, CXCR1, CXCR2, CXCR3, or CXCR5 [47-49], ATLL cells fit, if anything, Th2 cell character. This, however, does not agree with the hypothesis obtained by the cytokine production profile that indicated Th1 cell origin. Again, it may not be appropriate to apply the Th1-Th2-dichotomy to the origin of lymphoid neoplasms, or the Th1-Th2-dichotomy may not correlate with chemokine receptor expression in humans as it has been reported that chemokine receptors are not significantly different between Th1 and Th2 cells in normal human peripheral blood [50].

Although the Th1-Th2-dichotomy is thus unclear in ATLL cells, the expressed chemokine receptors are functional and do correlate with clinical manifestations. CCR7 is necessary for the homing of naïve and memory T cells to secondary lymphoid organs through the gateway of high endothelial venules [51]. ATLL cells express CCR7, and migrate toward CCR7 chemokines, CCL19 (EBI1-ligand chemokine: ELC), and CCL21 (secondary lymphoid-tissue chemokine: SLC) [47]. Consequently, ATLL cells from patients with lymphoid organ involvement express significantly higher levels of CCR7 than control CD4+CD45RO+ T cells or ATLL cells from patients

without lymphoid organ involvement [47].   CCR4 is selectively expressed by cutaneous lymphocyte-associated antigen (CLA)-positive skin-homing memory T cells.   ATLL cells from almost all patients express CCR4 and show vigorous migration toward its ligands, CCL17 (thymus and activation-regulated chemokine: TARC), and CCL22 (macrophage-derived chemokine: MDC) that are produced from epidermal keratinocytes [48]. Moreover, ATLL skin lesions contain abundant transcripts of CCR4, CCL17, and CCL22, indicating a significant role for CCR4 expression in skin invasion.   Another skin-homing chemokine receptor CCR10, which is expressed in a fraction of CLA+ T cells [52, 53], is also expressed in ATLL cells, and ATLL cells migrate toward its ligand, CCL27 (cutaneous T-cell attracting chemokine: CTACK) [49].   These results indicate that ATLL cells have a high affinity for the skin.   In fact, a variety of skin lesions, erythema, papules, and nodule- or tumor-formations are observed in more than one third of ATLL patients from the first diagnosis.   Another unique characteristic is that ATLL cells not only express CCR8 but also produce its ligand CCL1 (I-309) [54].   Since CCL1 inhibits the apoptosis of ATLL cells, an anti-apoptotic autocrine mechanism is suggested [54].

    4. Other cell surface markers (Table 2)

Fas (APO-1/CD95) is a member of the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily. Cross-linking of cell membrane Fas (mFas) by Fas ligand (FasL) or by agonistic IgM anti-Fas monoclonal antibodies transduces a death signal, producing apoptosis [55]. Primary ATLL cells express mFas [56], which is a characteristic of activated CD45RO+ T-cells. In spite of its expression, ATLL cells are usually resistant to Fas-medicated apoptosis [57]. Since the Fas ligand is produced daily as a result of immune reactions, ATLL cells should have been exposed to the FasL *in vivo*. ATLL cells have protection mechanisms against FasL. One of these mechanisms is the production of soluble Fas (sFas), which lacks a transmembrane domain and captures and inactivates FasL. ATLL cells secrete sFas, and the sera from ATLL patients contain significantly higher concentrations of sFas than sera from healthy adults [58, 59]. Another mechanism is a function ablating Fas gene mutation that loses the ability to transduce the death signal, and ATLL patients with such mutations have been reported [60, 61]. In addition, HTLV-1 Tax protein confers resistant mechanisms on ATLL cells at multiple levels towards the apoptosis cascade [62].

TNF-related apoptosis-inducing ligand (TRAIL) induces apoptosis in

many transformed cells, but not in normal cells, and hence, TRAIL has recently emerged as a novel anti-cancer agent. Although primary ATLL cells express the TRAIL-death receptors, DR4 and DR5, they are usually resistant to TRAIL exposure [63]. The resistance mechanism is complex and is not explained by a single factor, but HTLV-1 Tax, at least in part, seems to be involved in this mechanism [63].

Regulatory T cells (Treg cells)

More than 30 years ago, two groups proposed the concept of suppressor T cells [64-66]. They aimed to disclose the suppression mechanism and asserted that the activity was mediated by antigen-specific suppressor cells. They, however, ceased this assertion by the middle of 1980s and stopped using the term "suppressor T cells", mostly because they had failed to clone suppressor factors and to identify the responsible gene I-J locus [67-70]. In a different stream of immune suppression study, Nishizuka and Sakakura showed that neonatal thymectomy in normal mice resulted in the development of an autoimmune disease [71]. Later, Sakaguchi *et al.* showed that depletion of Lyt-1 (CD5) cells from adult mice resulted in the development of autoimmune diseases, whereas depletion of Lyt-2 (CD8) cells

did not, suggesting the existence of suppressor T cells in the CD5+CD8- cell population [72]. They, thereafter, confirmed that the suppressor cells (currently termed Treg cells) existed in the CD4+ T cell fraction [73]. Interestingly, the suppressor activity was mediated by CD4+ T cells characterized by CD25 expression; when CD4+ cell suspensions were depleted of CD25+ cells and then inoculated into BALB/c athymic nude (nu/nu) mice, all recipients spontaneously developed several kinds of autoimmune diseases [73]. The reconstitution of CD4+CD25+ cells within a limited period after the transfer of CD4+CD25- cells prevented the development in a dose dependent manner. Of note, the depletion of CD4+CD25+ T cells enhanced immune responses not only against self-antigens but also against non-self antigens including soluble xenogeneic proteins and allografts. They therefore concluded that CD4+CD25+ cells contribute to maintaining self-tolerance by down-regulating the immune response to self and non-self antigens in an antigen-nonspecific manner, presumably at the T cell activation stage. The suppressive activity of CD4+CD25+ cells has also been confirmed *in vitro*, and CD4+CD25+ cells suppressed the proliferation of anti-CD3- and concanavalin A (ConA)-induced CD4+CD25- T cells proliferation, the mechanism of which

was dependent on the manner of cell-to-cell contact and did not require soluble factors [74, 75].

In 2001, Foxp3, a member of the forkhead/winged-helix family of transcriptional regulators, was identified to be the causative gene of scurfy mice that are characterized by overproliferation of CD4+CD8- T cells, extensive multiorgan infiltration, and the elevation of numerous cytokines such as IL-4, IL-6, IL-7, and TNF-α [76, 77]. IPEX (immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome) is a human counterpart of scurfy mice and is caused by different mutations of Foxp3 [78]. Foxp3 is specifically expressed in naturally arising Treg cells in mice, furthermore, retroviral gene transfer of Foxp3 converts naïve T cells toward a Treg phenotype similar to that of naturally occurring Treg cells, indicating that Foxp3 is the master controller of Treg cell development and function [79]. There are, however, some differences between mouse and human Foxp3, and it has been reported that human CD4+ T cells with ectopically overexpressed Foxp3 do not acquire significant suppressor activity *in vitro* [80]. Moreover, Foxp3 mRNA expression could be induced not only in CD4+CD25- but also in CD8+ peripheral blood T cells in humans by cell activation [81]. Conversely, IL-10-producing regulatory T cells (Tr1) do not

constitutively express Foxp3 although they do exhibit suppressive activity [82]. Thus, Foxp3 is a poor marker of regulatory T cells in humans.

Several other molecules are also known to be associated with Treg cell function. Treg cells express CTLA-4 that is essential in T cell-mediated dominant immunologic self-tolerance [83, 84], and *in vivo* blockade of CTLA-4 in normal mice leads to the spontaneous development of autoimmune diseases. Treg cells express high and persistent levels of TGF-β on their cell surface, which is also essential for their functioning *via* cell-to-cell contact [85]. Anti-TGF-β antibody not only abrogates the suppression of CD4+CD25- T cell proliferation but also the suppression of B cell differentiation and immunoglobulin production by Treg cells. GITR, a member of the tumor necrosis factor-nerve growth factor receptor family, is expressed on Treg cells, and the removal of GITR-expressing T cells produces organ-specific autoimmune disease in mice [86]. OX40 (CD134) and 4-1BB (CD137) are also expressed in Treg cells. However, the specificity of these markers in Treg cells is controversial because they are up-regulated in non-Treg cells following activation. As for chemokine receptor expression, it has been reported that human Treg cells display a restricted expression profile, namely CCR4+ and CCR8+, and CCR8 expression is more restricted

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to Treg cells [87, 88].   The expression of CCR5, CCR6, and CCR7 in Treg cells has also been shown in murine systems.   Nowadays, there is a large body of evidence concerning the implications of Tregs in many immune processes such as tumor evasion, autoimmunity, and pathogen elimination. There are also numerous clinical trials being conducted in many parts of the world with the aim of using these cells as therapeutic agents.

ATLL as a regulatory T-cell tumor

For a long time, the focus of the intensive research into T cell function has been the regulation of B cell differentiation, and this research was profoundly facilitated by the development of monoclonal antibodies against T cell subsets.   It was commonly accepted that the CD4+ T cell subset possessed helper activity and the CD8+ T cell subset possessed suppressive activity, and that CD4+ T cells served to induce the suppressive activity of CD8+ T cells [89, 90].   In 1978, Uchiyama *et al.* showed for the first time that primary ATLL cells exhibited a strong suppressive activity on pokeweed mitogen (PWM)-induced normal B-cell differentiation [91].   Other investigators also confirmed the activity, but the interpretation of the mechanism was conflicting among investigators; some asserted that ATLL

cells induced the suppressive activity of CD8+ T cells, but others asserted that ATLL cells directly acted as suppressors [92-95]. Since primary ATLL cells exhibit suppressive activity without the participation of normal CD8+ T cells, moreover, the effect is lost by radiation of ATLL cells but not of normal CD8+ cells [94, 95], it seems natural to think that ATLL cells themselves exhibit suppressive activity. Importantly, the activity was mediated in a cell-to-cell contact-dependent manner, similar to the activity of Treg cells, and the supernatant of the ATLL cell culture did not contain suppressive activity [95]. The production of IL-10 and TGF-β from ATLL cells, the two major cytokines reported to be involved in Treg cell function, was disclosed later [96, 97].

After the discovery of the Foxp3 gene in 2003 [79], many investigators reexamined ATLL cells from the viewpoint of Treg cells [98-103]. These studies revealed that ATLL cells indeed expressed Foxp3 although the expression level differed greatly among patients. In the immunohistochemical analysis of 172 paraffin-embedded lymphoma samples, Foxp3 expression was confined to the ATLL cases, and no other lymphoma types exhibited Foxp3 expression [100]. ATLL cells also express other Treg-cell markers. ATLL cells express significantly higher levels of GITR

mRNA than normal T cells, and HTLV-1 transactivator Tax induces GITR expression [99]. Since Tax, in contrast, does not transactivate the Foxp3 gene [99], Foxp3 expression is undoubtedly an innate characteristic of ATLL cells. ATLL cells express CTLA-4 and the expression correlates with Foxp3 mRNA levels [102]. The expression of OX40 in ATLL cells was shown long time ago, and was interpreted to be involved in leukemic cell infiltration [104]. In recent functional analyses of ATLL cells, it was confirmed that ATLL cells inhibit Concanavalin A- and anti CD3 antibody-stimulated T cell proliferation in a dose dependent manner not only in an allogeneic setting but also in autologous setting [99, 101-103]. The activities were not abrogated by antibodies against TGF-β or IL-10, which excludes the participation of these molecules in the suppression mechanism [101]. The lack of IL-2 production is also one of the key markers for Treg cells [73, 74], and primary ATLL cells and ATLL cell lines never produce IL-2 after any stimulation including Ca-ionophore A23187 with ionomycin, phorbolmyristate acetate (PMA), and Con A [28, 102].

Since the retroviral transfection of Foxp3 does not confer any suppressive function on low Foxp3-expressing HTLV-1-infected cell lines, it is assumed that Foxp3 is essential but is not sufficient for Treg-cell-like suppressive

activity [101]. In other words, the suppressive activity of ATLL cells comes from their innate characteristics but not from Foxp3 expression. In this aspect, it is interesting that, besides ATLL and HTLV-1-infected cell lines, Foxp3 mRNA was also detected in many kinds of human leukemia and transformed cell lines, although Foxp3 protein expression was limited in ATLL cell lines [105]. Moreover, a variety of primary human cancer cells also express Foxp3 [106, 107]. It is thus still debated at least in humans whether Foxp3 is an exclusive marker for Treg cells. Recent publications have disclosed the novel role of Foxp3. Foxp3 is expressed in mammalian epithelial cells and acts as a transcriptional repressor of oncogenes such as SKP2 and HER-2/ErbB2 [108, 109]. Deletion, functionally significant somatic mutations, and downregulation of the Foxp3 gene are commonly found in human breast cancer samples and correlate significantly with HER-2/ErbB2 overexpression [108].

Another difficulty for identifying Treg cells is that the molecule(s) responsible for the direct suppressive activity remains largely unknown. It has recently been reported that Epstein-Barr-virus-induced gene 3 (*Ebi3*, which encodes IL-27β) and IL-12α (*IL-12α*, which encodes IL-12α/p35) are highly expressed by mouse Foxp3+ Treg cells but not by resting or activated

effector CD4+ T cells (Teff), and that an Ebi3-IL-12α heterodimer is constitutively secreted by Treg cells but not Teff cells [110]. This novel Ebi3-IL-12α heterodimeric cytokine has been designated IL-35 [111], and the ectopic expression of IL-35 confers regulatory activity on naïve T cells, moreover, recombinant IL-35 suppresses CD4+CD25- Teff cell proliferation [107, 110]. IL-35 also shows the activity in an *in vivo* setting, and attenuated established collagen-induced arthritis in mice [110]. These data identify IL-35 as a novel inhibitory cytokine that may be specifically produced by Treg cells. There is, however, no available data about IL-35 expression in human cells including ATLL cells.

Conclusion

ATLL cells satisfy almost all the criteria for natural Treg cells; ATLL cells are CD4+CD25+, express Foxp3, CTLA-4, GITR, OX40, CCR4, and CCR8, and produce IL-10 and TGFβ, but not IL-2. Furthermore, immunobiological analysis of ATLL cells *in vitro* and the *in vivo* profile of ATLL patients both support the assertion that ATLL is a neoplasm of Treg cells. So, we wonder what we need to do next to confirm this theory. Perhaps an examination of IL-35 would be useful.

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Figure legends:

Fig. 1. Peripheral blood ATLL cells. Typical ATLL cells with multi-lobulated nuclei, so called flower cells, are shown.

Fig. 2. Southern blot analysis of HTLV-1 proviral DNA in primary leukemia samples. Cellular DNA was digested with *Eco*R1 (E) or *Pst*1 (P), and hybridized with an HTLV-1 probe. Since HTLV-1 does not have an *Eco*R1 site but does have *Pst*1 sites, the presence of band(s) in *Eco*R1-digested DNA indicate the monoclonal proliferation of HTLV-1 integrated cells (ATLL 1 and ATLL 2). In *Pst1*-digested DNA, in contrast, three internal bands of 2.4bp, 1.6bp and 1.1bp (arrow heads) in length are observed even without the presence of monoclonal proliferation, if HTLV-1 infected cells are more than 5%. The presence of band(s) other than these three bands indicates the presence of monoclonal proliferation, and the loss of these bands indicates that the provirus is a defective virus. M: size marker. CL: positive control bands obtained by an ATLL cell line.
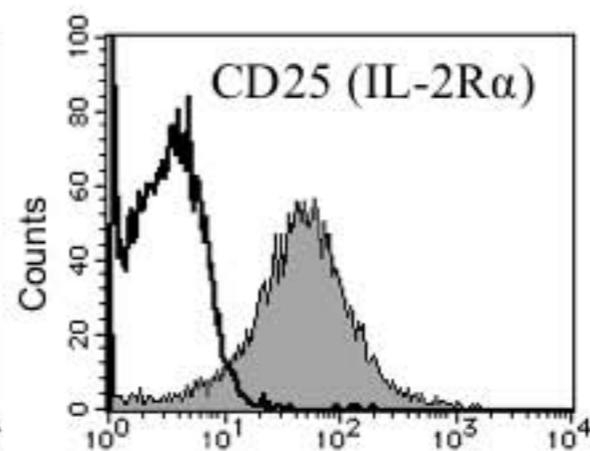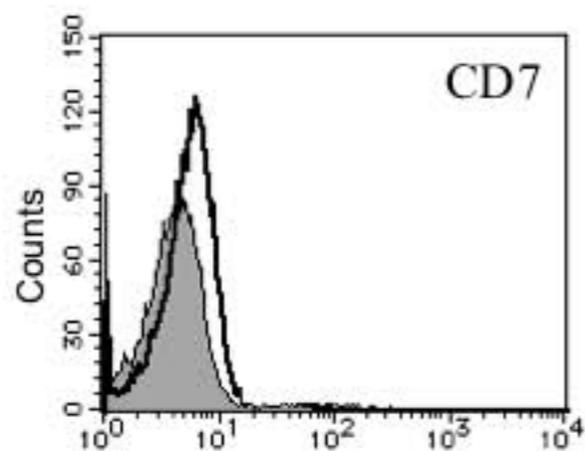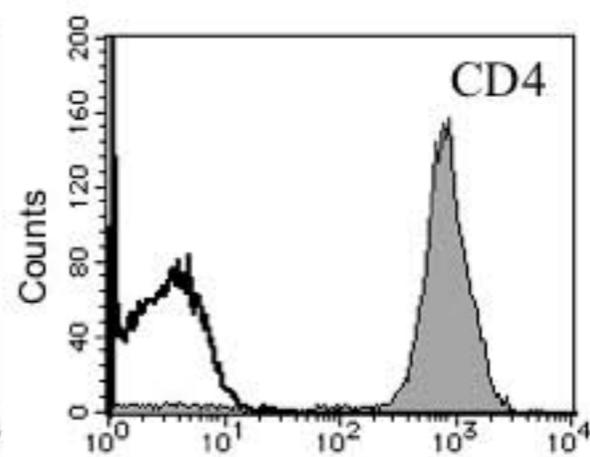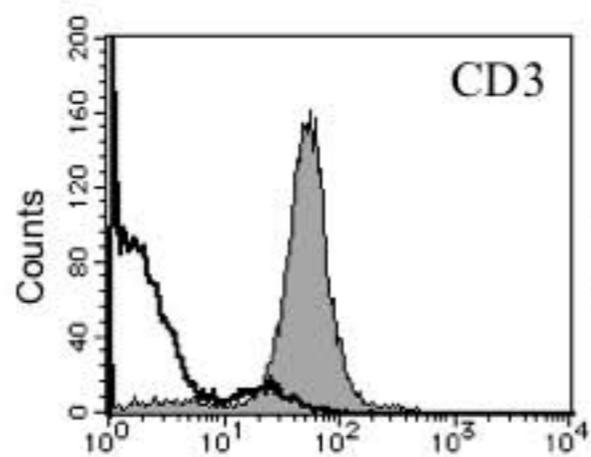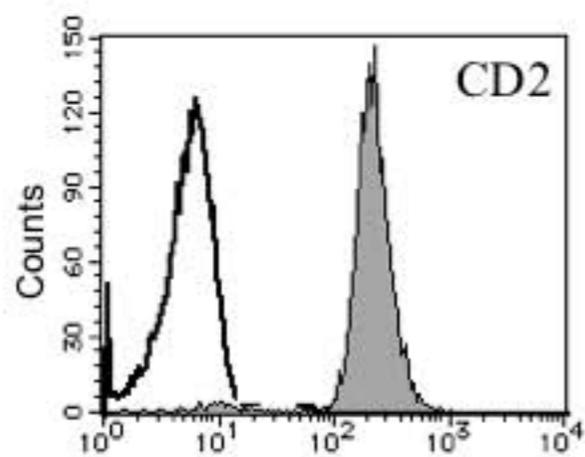
Fig. 3. Surface phenotype of ATLL cells. ATLL cells are CD2+, CD3+, CD4+,

CD5+, CD7-, CD8- and express all members of the γc-receptor family except for IL-9R (IL-4R+, IL-7R+, IL-15R+, and IL-21R+).
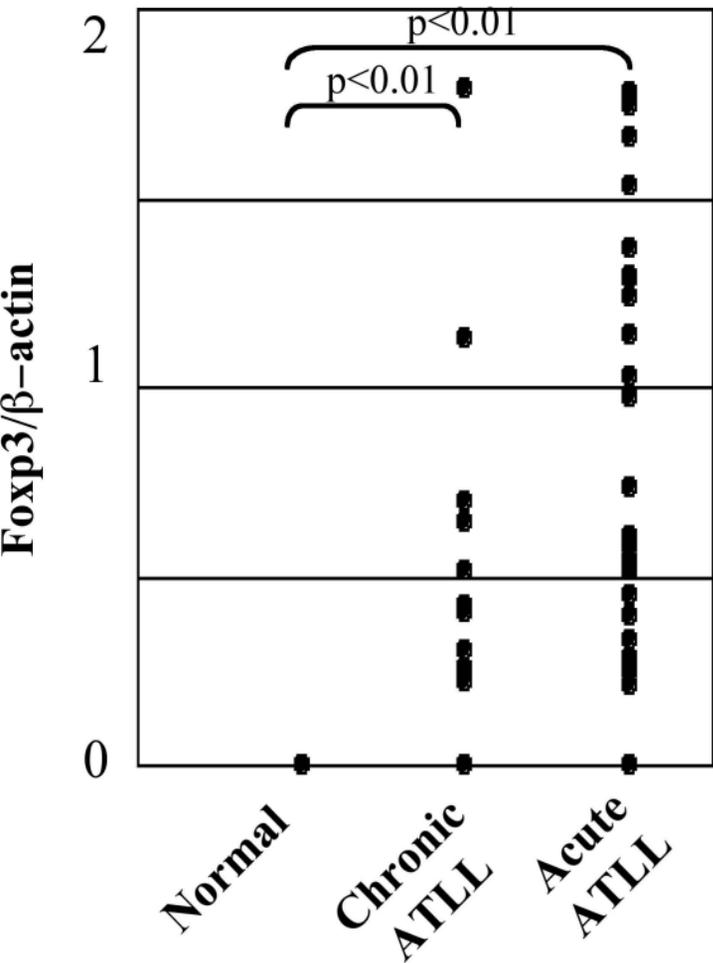
Fig. 4. Foxp3 and GITR expression in ATLL cells.　Quantitative analyses by real-time RT-PCR show a significant elevation of Foxp3 and GITR mRNAs in primary ATLL cells.　Modified from Ref. no. [99].
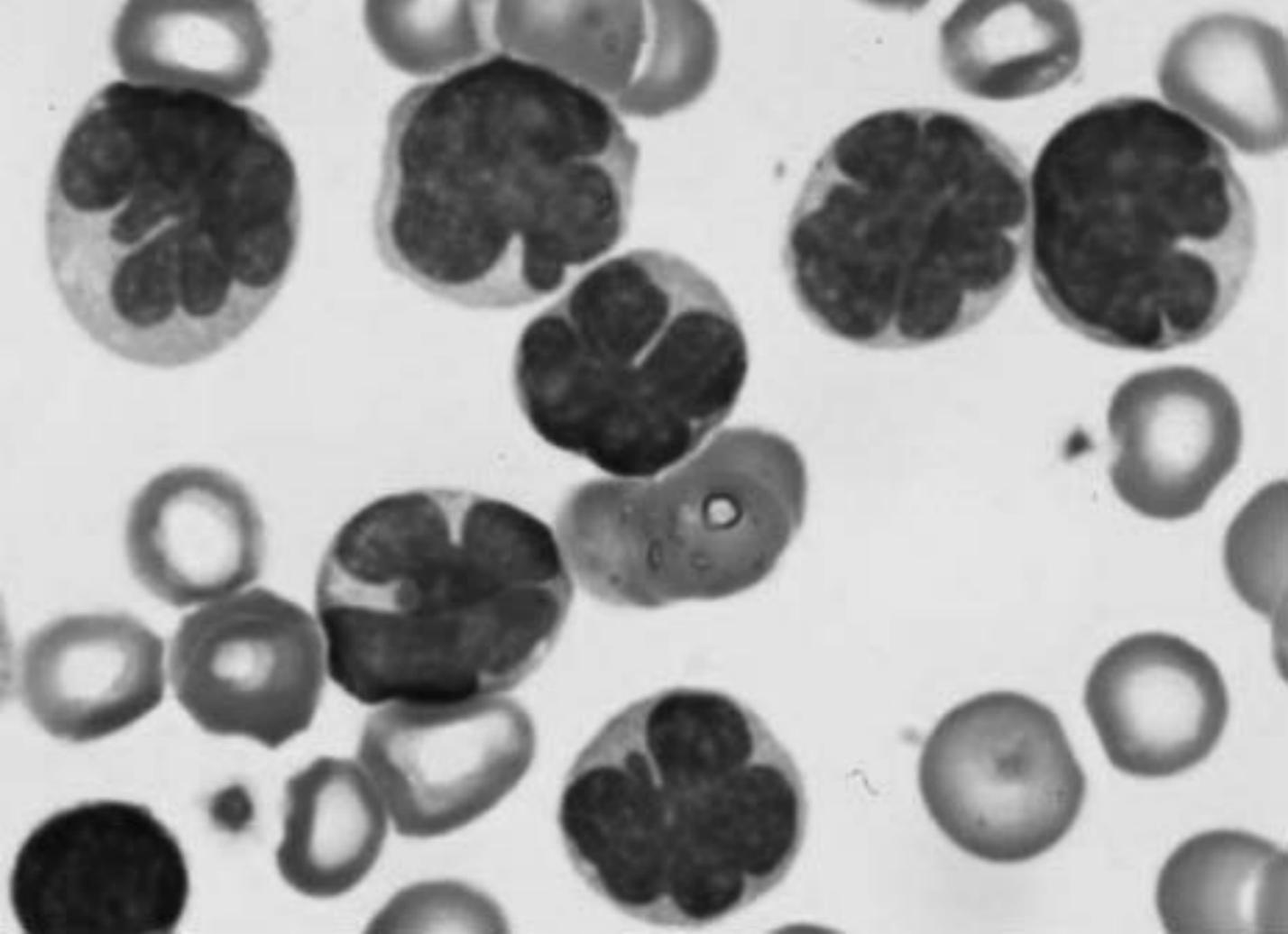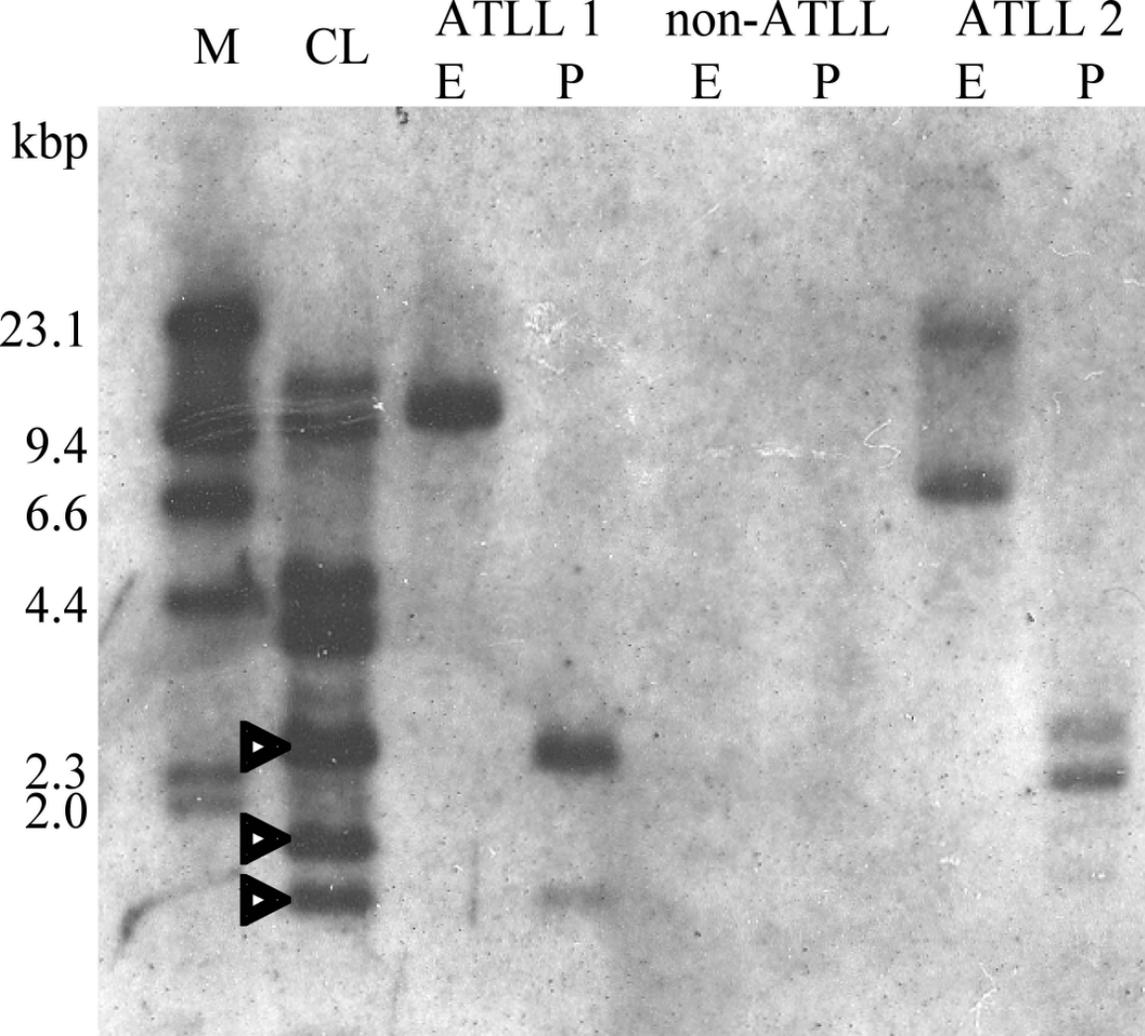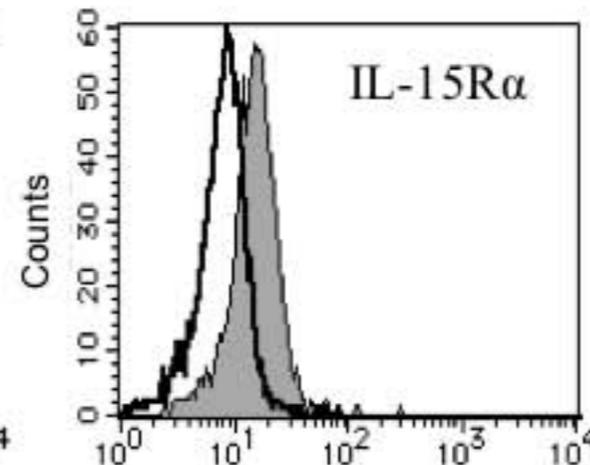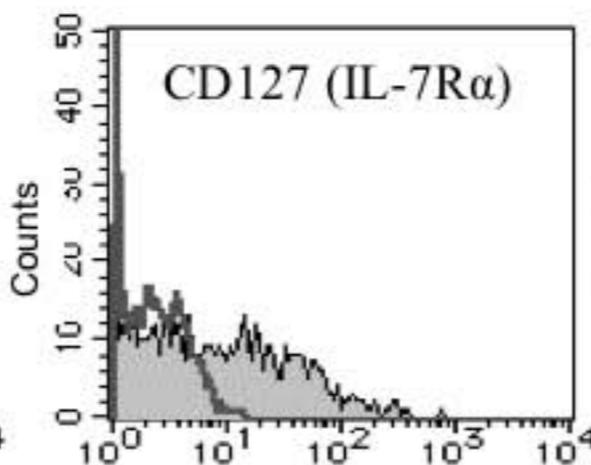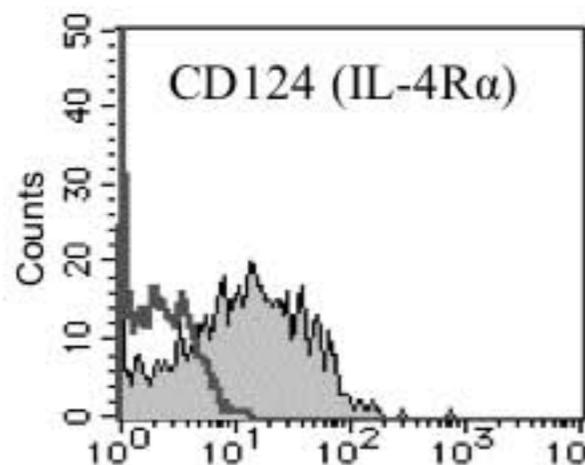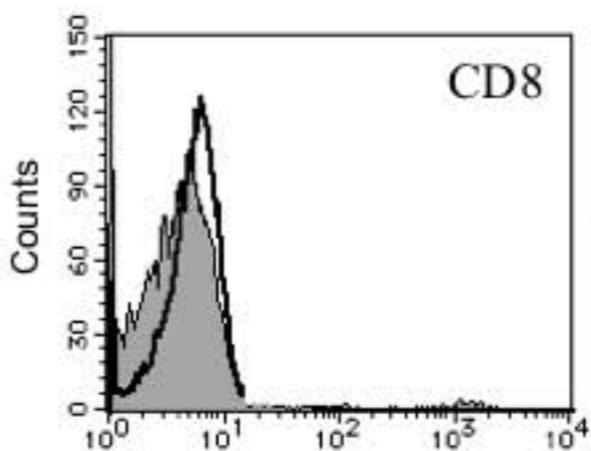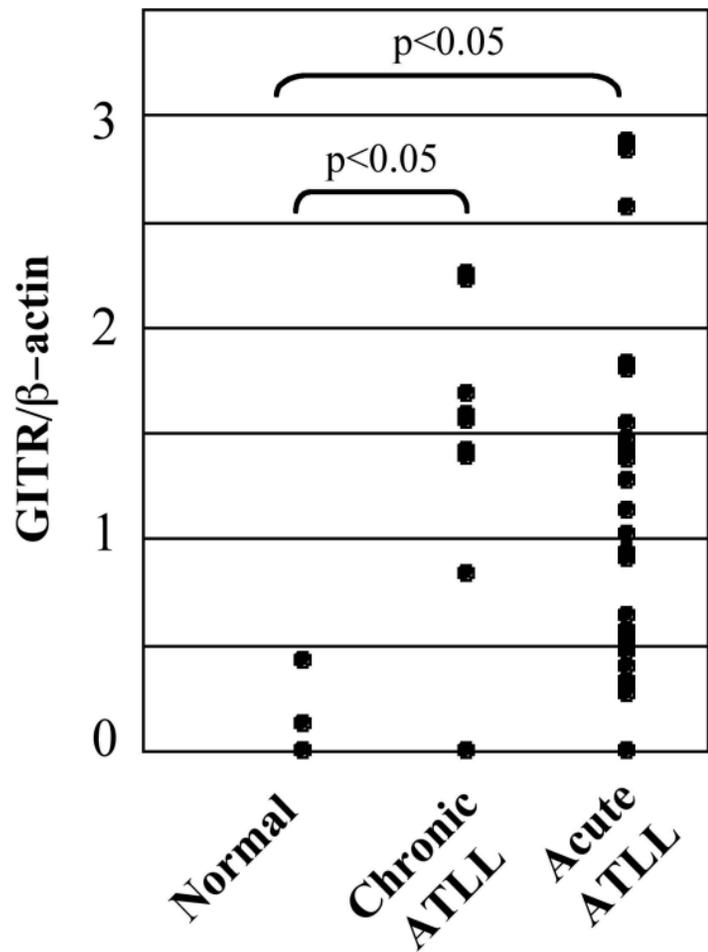
Table 1. Episodes of documented infections during admission (modified from Ref. no. [9])

	ATLL (n=112)	NHL (n=109)	p
Bacterial pneumonia	23 (5)	10 (2)	< 0.05
Sepsis	6 (1)	3	
Skin infections	4 (1)	0	< 0.05
Tuberculosis	3 (3)	0	
Pneumocystis jiroveci pneumonia	5 (3)	0	< 0.05
Fungal infections	16 (1)	5	< 0.05
Cytomegalovirus infection	9	1	< 0.05
Herpes zoster	12 (2)	6	
Herpes simplex virus infection	9	0	< 0.01

( ): number of infectious episodes before chemotherapy

NHL: non-Hodgkin lymphoma

Table 2. Immunological characteristics of ATLL cells

T cell-related markers	
Positive	CD2, CD3, CD4, CD5, CD30, CD45RO, TCRα/β
Negative or very low	CD1, CD7, CD26, CD45RA, TCRγ/δ
	(some cases show CD2-, CD5-, CD4+CD8+, CD4-CD8-, CD4-CD8+ and/or CD45RA+ phenotypes)
Cytokine production	
Positive	IFNγ, IL-6, IL-8, LD78/MIP-1α, TNF-α, M-CSF, GM-CSF, VEGF
Negative	IL-1α, IL-1β, IL-2, IL-4, IFNα, G-CSF
Cytokine receptors	
Positive	IL-2Rα(CD25), IL-2Rβ, IL-2Rγ, IL-4R, IL-7R, IL-15R, IL-21R
Chemokine receptors	
Positive	CCR1, CCR4, CCR7, CCR8, CCR10, CXCR4
Negative	CCR2, CCR3, CCR5, CCR6, CXCR1, CXCR2, CXCR3, CXCR5

Table 3. Comparison of human Treg cells and ATLL cells

	Human Treg cells	ATLL cells
Cell surface markers	CD4+, CD8-, CD45RO+	CD4+, CD8-, CD45RO+
Cytokine production		
Positive	IL-10, TGFβ	IL-10, TGFβ
Negative	IL-2	IL-2
Cytokine receptors		
Positive	IL-2Rα (CD25)	IL-2Rα (CD25)
Chemokine receptors		
Positive	CCR4, CCR8	CCR4, CCR8
Treg markers		
Positive	Foxp3, GITR, CTLA-4, OX40	Foxp3, GITR, CTLA-4, OX40
Function		
in vitro	Suppress CD4+CD25- T cell proliferation	Suppress T cell proliferation and B cell differentiation
in vivo	Develop autoimmune diseases if absent	Patients are in a severely immune suppressed condition