or cancer antigen in association with major histocompatibility complex (MHC) class I molecules and by suppressing viral replication and tumor development via IFN-γ secretion. Elucidating the role of HTLV-1–specific $CD8^+$ CTLs has been considered a priority issue in studies of host defense mechanisms involved in HTLV-1 infection (Bangham, 2008; Jacobson, 2002; Kannagi, 2007).

3.1 HTLV-1-specific cytotoxic T lymphocytes

T-cell receptors (TCR) on CTLs recognize peptide fragments derived from viral and tumor antigens that are presented on MHC class I molecules by antigen-presenting cells or virus-infected cells. After TCR binds to the peptide-MHC complex, CTLs are activated and fulfill an effector function. There are 3 main effector mechanisms by which the $CD8^+$ CTL kills virus-infected or tumor cells. One is to release perforin and granzymes. Perforin forms pores in the plasma membrane of the target cells, allowing entry of granzymes; caspases are then activated, leading to apoptosis. Apoptosis may also be induced via a Fas-FasL interaction between CTLs and target cells. Finally, $CD8^+$ cells can produce IFN-γ, which has indirect cytolytic effects by promoting NK cell activity and macrophage activation.

The Tax protein is an immunodominant antigen in HTLV-1 infections. Therefore, CTL activity is predominantly restricted to products of the HTLV-1 Tax gene, although HTLV-1 Env, Pol, Rof, Tof, and HBZ (Elovaara et al., 1993; Hilburn et al., 2011; Macnamara et al., 2010; Pique et al., 2000) could also be target proteins of HTLV-1-specific CTL. In a study that utilized properties of the CTL antigen recognition system, human MHC class I HLA-A2(*0201) tetramers loaded with HTLV-1 Tax peptide were used to detect HTLV-1 Tax specific HLA-A2-restricted $CD8^+$ cells (Bieganowska et al., 1999, Greten et al., 1998). This technique facilitates quantification of the frequency of antigen-specific T cells, as well as direct characterization of these cells. HLA genotype determines which part of the viral protein is presented as an antigen peptide. For HLA-A*0201 and HLA-A*2402, for example, the major epitopes are the Tax 11-19 and Tax 301-309 amino acids, respectively.

3.2 Abnormal CTL response in patients with ATL

An increasing number of studies in patients with HTLV-1-associated disorders have documented an association between the disorders and abnormalities in both the frequency of CTLs and their response to HTLV-1. When peripheral blood mononuclear cells (PBMCs) from HTLV-1 carriers are stimulated with autologous HTLV-1–infected cells *in vitro*, proliferation of HTLV-1-specific $CD8^+$ CTLs is often observed in the presence of IL-2. An increased level of HTLV-1–specific CTL responses occurs in all HAM/TSP patients and in some asymptomatic HTLV-1 carriers; however, HTLV-1–specific CTL responses are rarely induced in PBMC cultures from ATL patients (Jacobson et al., 1990; Kannagi et al., 1984, Parker et al., 1992). HTLV-1–specific CTLs are also present in ATL patients but do not expand sufficiently (Arnulf et al., 2004). Impairment of the HTLV-1 specific CTL response was observed in some individuals during the earlier stages of HTLV-1 infection (AC and smoldering ATL), as well as in advanced ATL patients (Shimizu et al., 2009). This observation suggests that the T-cell insufficiency in ATL patients is present prior to disease onset. In addition, a recent report indicated that, in comparison to ACs, ATL patients have a smaller and less diverse population of HTLV-1 specific $CD8^+$ T cells, as well as lower anti-HTLV-1 $CD8^+$ T cell expression of perforin and granzyme B (Kozako et al., 2006). Thus, the decreased number and functional impairment of CTLs might contribute to the onset and progression of ATL.

Furthermore, Tax-specific CTL responses were strongly activated in some ATL patients who achieved complete remission after hematopoietic stem cell transplantation (HSCT), but were not observed in the same patients before transplantation (Harashima et al., 2004). This suggests that HTLV-1-specific CTLs, including Tax-specific CTLs, play an important role in surveillance against HTLV-1 leukemogenesis.

3.3 Abnormal CTL response in patients with HAM/TSP

One of the most striking features of the adaptive immune system in HAM/TSP patients is the larger number of HTLV-1-specific $CD8^+$ CTLs (Elovaara et al., 1993; Greten et al., 1998; Jacobson et al., 1990; Kubota et al., 2002; Nagai et al., 2001a; Parker et al., 1992). While HTLV-1 specific CTLs are also detectable in the PBMC of ACs (Parker et al., 1992), the magnitude and frequency of these responses are clearly higher in patients with HAM/TSP, particularly in the CSF (Elovaara et al., 1993; Nagai et al. 2001a). In addition, the HTLV-1 proviral load of HAM/TSP patients may be 5- to 16-fold higher than that of ACs (Hashimoto et al., 1998; Kubota et al., 1993; Nagai et al., 1998). While some studies have found a positive correlation between the frequency of HTLV-1-specific $CD8^+$ T cells and HTLV-1 proviral load has been detected in PBMCs from HAM/TSP patients (Kubota et al., 2000, Nagai et al., 2001b, Yamano et al., 2002), this result is not ubiquitous (Wodarz et al., 2001). Thus, the cytolytic activity of CTLs, rather than their frequency, might be impaired in HAM/TSP patients.

There are some methods to measure CTL cytolytic activity. One is the sensitive CD107a mobilization assay, which quantifies the amount of lysosomal membrane protein LAMP-1 (CD107a) present on the CTL surface (CD107a) (Betts et al. 2003). Among studies that have used this method to evaluate CTL function, results are conflicting; while one reported that HTLV-1-specific CTLs of HAM/TSP patients had significantly lower CD107a staining than those of ACs (Sabouri et al., 2008), another study reported the opposite (Abdelbary et al., 2011). Furthermore, higher expression of CD107a/IFN-γ was induced by tax peptide stimulation in the $CD8^+$ T cells of HAM/TSP patients than in those of ACs (Enose-Akahata et al., 2008). Thus, it is not yet clear whether the cytolytic activity of HTLV-1-specific CTL in HAM/TSP patients is insufficient. However, these findings suggest that quantity of HTLV-1-infected cells is not determined by HTLV-1-specific CTL alone; additional factors, such as innate immunity and the proliferative ability of infected cells, must be relevant.

3.4 Pathogenic Role of CTL in HAM/TSP

In HAM/TSP patients, HTLV-1-specific $CD8^+$ CTL levels are extraordinarily high in peripheral blood, and even higher in cerebrospinal fluid (CSF) (Elovaara et al., 1993; Greten et al., 1998; Jacobson et al., 1990; Kubota et al., 2002; Parker et al., 1994; Nagai et al., 2001; Yamano et al., 2002). Immunohistochemical analysis of affected spinal cord lesions in early-stage HAM/TSP patients revealed the presence of infiltrating $CD4^+$ and $CD8^+$ lymphocytes, among which $CD8^+$ cells become increasingly dominant over the duration of the illness (Umehara et al., 1993). The expression of HLA class I antigens (Moore et al., 1989) and the existence of HTLV-1 specific $CD8^+$ CTLs have also been found in such lesions (Levin et al., 1997). In addition, the infiltration of $CD8^+$ CTLs in the affected spinal cord was characterized as positive for TIA-1 that is a marker of CTL (Umehara et al. 1994, Anderson et al. 1990). The number of TIA-1^+ cells was clearly related to the amount of the proviral DNA *in situ*, and the number of infiltrating $CD8^+$ cells appears to correlate with the presence of apoptotic cells.

Tax-specific $CD8^+$ CTL clones secrete various inflammatory cytokines, chemokines, and matrix metalloproteinases (MMP), such as IFN-γ, TNF-α, monocyte inflammatory protein (MIP)-1α, MIP-1β, interleukin(IL)-16, and MMP-9 (Biddison et al., 1997). TNF-α induces cytotoxic damage to endothelial cells, thus decreasing the integrity of the blood-brain barrier. It can also directly injure oligodendrocytes. MIP-1α and 1β can enhance transendothelial migration of lymphocytes into the central nervous system. IL-16 is a chemoattractant for $CD4^+$ cells, which are the major source of IL-2 required by IL-2 non-producer $CD8^+$ cells for proliferation. Therefore, HTLV-1-specific $CD8^+$ CTLs are an important source of proinflammatory soluble mediators that may contribute significantly to the pathogenesis of HAM/TSP. These observations continue to support the hypothesis that HTLV-1-specific $CD8^+$ CTLs are a major contributing factor in the immunopathogenesis of HAM/TSP.

4. Abnormality of innate immunity

Besides CTLs, there are several cell populations in the human immune system that have cytolytic activity against virus-infected cells, including natural killer (NK) cells, natural killer T (NKT) cells, and γδ T cells, which are cellular components of innate immunity. Dendritic cells (DCs) play an important role in the activation of these cell populations and CTLs. There is little evidence suggesting a role for γδ T cells in the pathogenesis of HTLV-1-associated disorders. Thus, this section focuses solely on the roles of DCs, NK cells, and NKT cells in HTLV-1-associated diseases, by comparing with the role of these cells in HIV-1 infection.

4.1 Dendritic cells and HTLV-1

Immature DCs are located in peripheral tissues and can effectively capture antigens, leading to their maturation via the expression of MHC class I/II and co-stimulatory molecules such as CD80, CD86, and CD40. Mature DCs are professional antigen-presenting cells that are uniquely able to prime naïve T cells. There are 2 main subsets of DCs: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). These cells play important roles in the regulation of innate and adaptive immunity. mDCs can induce the activation of invariant NKT (iNKT) cells via surface expression of the CD1d/glycolipid complex. After antigen capture, pDCs secrete type 1 IFN, which induces the activation of NK cells and promotes the activation of iNKT cells by mDCs.
An *in vitro* study indicated that cell-free HTLV-1 effectively infects DCs, leading to the transmission and transformation of $CD4^+$ T cells (Jones et al. 2008). In addition to suggesting a mechanism for HTLV-1 transmission, this study also indicated that HTLV-1 infection of DCs plays a role in the pathogenesis of HTLV-1-associated disorders. In fact, HTLV-1-infected DCs are observed in the peripheral blood of HTLV-1-infected individuals (Hishizawa et al., 2004; Macatonia et al., 1992), and infected pDCs have an impaired ability to produce type I IFN (Azakami et al., 2009; Hishizawa et al., 2004). In addition, we recently reported that the frequency of mDCs and pDCs is significantly lower in patients with both HAM/TSP and ATL (Azakami et al., 2009). Cumulatively, these studies imply that decreases in the number and functionality of DCs interfere with innate immunity, thus leading to pathogenesis.

4.2 Natural killer cells and HTLV-1

NK cells are major components of the innate immune system and account for 10–15% of PBMCs in normal individuals. They have direct and indirect cytolytic activity against tumor

cells and virus-infected cells by producing perforins, granzymes, and IFN-γ. Human NK cells can be divided into 2 subsets on the basis of their cell-surface markers: $CD56^+CD16^+$ and $CD56^{bright}CD16^-$ NK cells. $CD56^+CD16^+$ NK cells are the major population of NK cells and have natural cytotoxic activity. $CD56^{bright}CD16^-$ NK cells are not cytotoxic but have the capacity to produce large amounts of IFN-γ upon activation. The activity of NK cells is regulated by a balance between positive and negative signals from different activating and inhibitory NK receptors. CD94/NKG2 receptor family is expressed on $CD8^+$ T cells and γδ T cells as well as NK cells, and is involved in the pathogenesis of HAM/TSP by modulating the activities of those cell populations (Saito et al. 2003, Mosley et al. 2005).
In both HIV-1- and HTLV-1-infected individuals, the number and function of NK cell subsets are impaired (Fortis et al., 2005). Multiple investigators have reported that the numbers of $CD56^+CD16^+$ NK cells in HAM/TSP and ATL patients are significantly lower than those observed in healthy controls (Azakami et al., 2009; Yu et al., 1991). Furthermore, NK cell activity was also lower in HAM/TSP patients than in healthy controls (Yu et al., 1991). When primary $CD4^+$ T cells are infected by HTLV-1, they can escape from NK cell-mediated cytotoxicity; HTLV-1 $p12^I$ downregulates the expression of intercellular adhesion molecule-1 (ICAM-1) and -2 on the surface of infected $CD4^+$ T cells, resulting in a reduced adherence of NK cells to HTLV-1-infected $CD4^+$ T cells (Banerjee et al., 2007).

4.3 Natural killer T cells and HTLV-1

Natural killer T (NKT) cells, a unique T cell subpopulation, constitute a subset of lymphocytes that share the features of innate and adaptive immune cells. Unlike conventional T cells, NKT cells express a TCR that recognizes glycolipids instead of protein antigens. Moreover, these cells share properties and receptors with NK cells. They rapidly produce granzymes and perforins upon stimulation. Among the $CD3^+$ T cells in human blood, 10–25% express NK cell surface molecules such as CD161, and these cells are classified as NKT cells. A small population of T cells within this NKT cell subset expresses a highly conserved Vα24Jα18 TCR chain that preferentially associates with Vβ11; these T cells are referred to as iNKT cells. Activation of human iNKT cells requires the presentation of glycolipids such as α-galactosylceramide (α-GalCer) on the MHC class I-like molecule CD1d. α-GalCer induces the rapid production of cytokines and potent antitumor and antipathogen responses by iNKT cells. $CD4^-$ iNKT cells preferentially induce the Th1 response and are more important than $CD4^+$ iNKT cells in controlling viral infection and cancer (Kim et al., 2002).
HIV-1-infected subjects have fewer iNKT cells in their peripheral blood than healthy donors (Sandberg et al., 2002; van der Vliet et al., 2002). The proliferative potential and INF-γ production of residual iNKT cells are impaired in HIV-1-infected individuals (Moll et al., 2009); likewise, patients with HTLV-1-associated disorders have a decreased frequency of iNKT cells in their peripheral blood (Azakami et al., 2009). Interestingly, in contrast to patterns observed in HIV-1 infections, HTLV-1 infection leads to preferential decreases of $CD4^-$ iNKT cells (Azakami et al., 2009). The production of perforin in iNKT cells is impaired in both ACs and HAM/TSP patients (Azakami et al., 2009). In addition, there is an inverse correlation between the frequency of iNKT cells and the HTLV-1 proviral load in the peripheral blood of HTLV-1-infected individuals (Azakami et al., 2009). Notably, *in vitro* stimulation of peripheral blood cells with α-GalCer leads to an increase in the number of iNKT cells and a subsequent decrease in the number of HTLV-1-infected T cells in samples

from ACs (Azakami et al., 2009). These results suggest that iNKT cells contribute to the immune defense against HTLV-1, and that iNKT cell depletion plays an important role in the pathogenesis of HAM/TSP and ATL.

5. Conclusion

Advances in our understanding of the immune system enhance studies of virus-host relationships. Although HTLV-1 causes 2 different diseases (ATL and HTM/TSP), $CD4^+CD25^+CCR4^+$ T cells are the common viral reservoir in both disorders. According to recent studies, however, characteristics of $CD4^+CD25^+CCR4^+$ T cells are completely different in the 2 diseases: Foxp3+ leukemic cells are found in ATL patients, while Foxp3- IFN-γ-producing cells are found in HAM/TSP patients. The host immune system plays a crucial role in controlling these HTLV-1-infected cells. HTLV-1-specific CTL is activated in patients with HAM/TSP, but not in those with ATL, indicating that impairment of acquired immunity is not universal. However, both ATL and HAM/TSP patients are known to experience decreases in innate immunity via the functional impairment of DCs, NK cells, and iNKT cells, as well as lower overall population numbers of these cell types. These conditions may contribute to inadequate viral control and play an important role in the pathogenesis of HTLV-1-associated disorders.

6. Acknowledgments

This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology; the Japanese Ministry of Health, Labor, and Welfare; the Uehara Memorial Foundation; the Nagao Takeshi Nanbyo Foundation; the Kanagawa Nanbyo Foundation; the Mishima Kaiun Memorial Foundation; the Takeda Science Foundation; the ITSUU Laboratory Research Foundation; the Foundation for Total Health Promotion; and the Sankyo Foundation of Life Science.

7. References

Abdelbary, N.H., Abdullah, H.M., Matsuzaki, T., Hayashi, D., Tanaka, Y., Takashima, H., Izumo, S. & Kubota, R. 2011. Reduced Tim-3 expression on human T-lymphotropic virus type I (HTLV-I) Tax-specific cytotoxic T lymphocytes in HTLV-I infection. *Journal of Infectious Diseases*, 203, 7, 948-959

Anderson, P., Nagler-Anderson, C., O'Brien, C., Levine, H., Watkins, S., Slayter, H.S., Blue, M.L. & Schlossman, S.F. 1990. A monoclonal antibody reactive with a 15-kDa cytoplasmic granule-associated protein defines a subpopulation of CD8+ T lymphocytes. *Journal of Immunology*, 144, 2, 574–582

Arnulf, B., Thorel, M., Poirot, Y., Tamouza, R., Boulanger, E., Jaccard, A., Oksenhendler, E., Hermine, O. & Pique, C. 2004. Loss of the ex vivo but not the reinducible CD8+ T-cell response to Tax in human T-cell leukemia virus type 1-infected patients with adult T-cell leukemia/lymphoma. *Leukemia*, 18, 1, 126-132

Asquith, B., Mosley, A.J., Barfield, A., Marshall, S.E., Heaps, A., Goon, P., Hanon, E., Tanaka, Y., Taylor, G.P. & Bangham, C.R. 2005. A functional CD8+ cell assay reveals individual variation in CD8+ cell antiviral efficacy and explains differences in

human T-lymphotropic virus type 1 proviral load. *Journal of General Virology*, 86, 5, 1515-23

Azakami, K., Sato, T., Araya, N., Utsunomiya, A., Kubota, R., Suzuki, K., Hasegawa, D., Izumi, T., Fujita, H., Aratani, S., Fujii, R., Yagishita, N., Kamijuku, H., Kanekura, T., Seino, K., Nishioka, K., Nakajima, T. & Yamano, Y. 2009. Severe loss of invariant NKT cells exhibiting anti-HTLV-1 activity in patients with HTLV-1-associated disorders. *Blood*, 114, 15, 3208-3215

Banerjee, P., Feuer, G., Barker, E. 2007. Human T-cell leukemia virus type 1 (HTLV-1) p12I down-modulates ICAM-1 and -2 and reduces adherence of natural killer cells, thereby protecting HTLV-1-infected primary CD4+ T cells from autologous natural killer cell-mediated cytotoxicity despite the reduction of major histocompatibility complex class I molecules on infected cells. *Journal of Virology*, 81, 18 9707-9717

Bangham, C.R. 2008. HTLV-1 infection: role of CTL efficiency. *Blood*, 112, 6, 2176-2177

Bangham, C.R. 2009. CTL quality and the control of human retroviral infections. *European Journal of Immunology*, 39, 7, 1700-1712

Betts, M.R., Brenchley, J.M., Price, D.A., De Rosa, S.C., Douek, D.C., Roederer, M. & Koup, R.A. 2003. Sensitive and viable identification of antigen-specific CD8+ T cells by a flow cytometric assay for degranulation. *Journal of Immunological Methods*, 281, 1-2, 65-78

Biddison, W.E., Kubota, R., Kawanishi, T., Taub, D.D., Cruikshank, W.W., Center, D.M., Connor, E.W., Utz, U. & Jacobson, S. 1997. Human T cell leukemia virus type I (HTLV-I)-specific CD8+ CTL clones from patients with HTLV-I-associated neurologic disease secrete proinflammatory cytokines, chemokines, and matrix metalloproteinase. *Journal of Immunology*, 159, 4, 2018-2025

Bieganowska, K., Hollsberg, P., Buckle, G.J., Lim, D.G., Greten, T.F., Schneck, J., Altman, J.D., Jacobson, S., Ledis, S.L., Hanchard, B., Chin, J., Morgan, O., Roth, P.A. & Hafler, D.A. 1999. Direct analysis of viral-specific CD8+ T cells with soluble HLA-A2/Tax11-19 tetramer complexes in patients with human T cell lymphotropic virus-associated myelopathy. *Journal of Immunology*, 162, 3, 1765-1771.

Boxus, M. & Willems, L. 2009. Mechanisms of HTLV-1 persistence and transformation. *British Journal of Cancer*, 101, 9, 1497-1501

Chen, S., Ishii, N., Ine, S., Ikeda, S., Fujimura, T., Ndhlovu, L.C., Soroosh, P., Tada, K., Harigae, H., Kameoka, J., Noriyuki, K., Sasaki, T. & Sugamura, K. 2006. Regulatory T cell-like activity of Foxp3+ adult T cell leukemia cells. *International Immunology* 18, 2, 269-277

Elovaara, I., Koenig, S., Brewah, A.Y., Woods, R.M., Lehky, T. & Jacobson, S. 1993. High human T cell lymphotropic virus type 1 (HTLV-1)-specific precursor cytotoxic T lymphocyte frequencies in patients with HTLV-1-associated neurological disease. *Journal of Experimental Medicine*, 177, 6, 1567-1573

Enose-Akahata, Y., Oh, U., Grant, C. & Jacobson, S. 2008. Retrovirally induced CTL degranulation mediated by IL-15 expression and infection of mononuclear phagocytes in patients with HTLV-Iassociated neurologic disease. *Blood*, 112, 6, 2400-2410

Fortis, C. & Poli, G. 2005. Dendritic cells and natural killer cells in the pathogenesis of HIV infection. *Immunologic Research*, 33: 1, 1-21

Gessain, A., Barin, F. & Vernant, J.C. 1985. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet*, 2, 8452, 407–410

Goon, P.K., Igakura, T., Hanon, E., Mosley, A.J., Barfield, A., Barnard, A.L., Kaftantzi, L., Tanaka, Y., Taylor, G.P. Weber, J.N. & Bangham, C.R. 2004. Human T cell lymphotropic virus type I (HTLV-I)-specific CD4+ T cells: immunodominance hierarchy and preferential infection with HTLV-I. *Journal of Immunology*, 172, 3, 1735–1743

Grant, C., Oh, U., Yao, K., Yamano, Y. & Jacobson, S. 2008. Dysregulation of TGF-beta signaling and regulatory and effector T-cell function in virus-induced neuroinflammatory disease. *Blood*,111, 12, 5601–5609

Greten, T.F., Slansky, J.E., Kubota, R., Soldan, S.S., Jaffee, E.M., Leist, T.P., Pardoll, D.M., Jacobson, S. & Schneck, J.P. 1998. Direct visualization of antigen-specific T cells: HTLV-1 Tax11-19- specific CD8(+) T cells are activated in peripheral blood and accumulate in cerebrospinal fluid from HAM/TSP patients. Proceedings of the National Academy of Sciences U.S.A., 95, 13, 7568–7573

Harashima, N., Kurihara, K., Utsunomiya, A. Tanosaki, R., Hanabuchi, S., Masuda, M., Ohashi, T., Fukui, F., Hasegawa, A., Masuda, T., Takaue, Y., Okamura, J. & Kannagi, M. 2004. Graft-versus-Tax response in adult T-cell leukemia patients after hematopoietic stem cell transplantation. *Cancer Research*, 64, 391–399

Hashimoto, K., Higuchi, I., Osame, M. & Izumo, S. 1998. Quantitative in situ PCR assay of HTLV-1 infected cells in peripheral blood lymphocytes of patients with ATL, HAM/TSP and asymptomatic carriers. *Journal of the Neurological Sciences*, 159, 1, 67–72

Hayashi, D., Kubota, R., Takenouchi, N., Tanaka, Y., Hirano, R., Takashima, H., Osame, M., Izumo, S. & Arimura, K. 2008. Reduced Foxp3 expression with increased cytomegalovirus-specific CTL in HTLV-I-associated myelopathy. *Journal of Neuroimmunology*, 200, 1-2, 115–124

Hieshima, K., Nagakubo, D., Nakayama, T., Shirakawa, A.K., Jin, Z., & Yoshie, O. 2008. Tax-inducible production of CC chemokine ligand 22 by human T cell leukemia virus type 1 (HTLV-1)-infected T cells promotes preferential transmission of HTLV-1 to CCR4-expressing CD4+ T cells. *Journal of Immunology*, 180, 2, 931-9

Hilburn, S., Rowan, A., Demontis, M.A., MacNamara, A., Asquith, B., Bangham, C.R. & Taylor, G.P. 2011. In vivo expression of human T-lymphotropic virus type 1 basic leucine-zipper protein generates specific CD8+ and CD4+ T-lymphocyte responses that correlate with clinical outcome. *Journal of Infectious Diseases*, 203, 4, 529-36

Hishizawa M, Imada K, Kitawaki T, Ueda M, Kadowaki N, Uchiyama T. 2004. Depletion and impaired interferon-alpha-producing capacity of blood plasmacytoid dendritic cells in human T-cell leukaemia virus type I-infected individuals. *British Journal of Haematology*, 125, 5, 568-575

Hori, S., Nomura, T. & Sakaguchi, S. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science*, 299, 5609, 1057–1061

Iwanaga, M., Watanabe, T., Utsunomiya, A., Okayama, A., Uchimaru, K., Koh, K.R., Ogata, M., Kikuchi, H., Sagara, Y., Uozumi, K., Mochizuki, M., Tsukasaki, K., Saburi, Y., Yamamura, M., Tanaka, J., Moriuchi, Y., Hino, S., Kamihira, S. & Yamaguchi, K. 2010. Human T-cell leukemia virus type I (HTLV-1) proviral load and disease

progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan. *Blood*, 116, 8, 1211-1219

Jacobson, S., Shida, H., McFarlin, D.E., Fauci, A.S. & Koenig, S. 1990. Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature*, 348, 6298, 245-248

Jacobson, S. 2002. Immunopathogenesis of human T cell lymphotropic virus type I-associated neurologic disease. *Journal of Infectious Diseases*, 186 Suppl, S187-92

Jones, K.S., Petrow-Sadowski, C., Huang, Y.K., Bertolette, D.C. & Ruscetti, F.W. 2008. Cell-free HTLV-1 infects dendritic cells leading to transmission and transformation of CD4(+) T cells. *Nature Medicine*, 14, 4, 429-436

Kannagi, M., Sugamura, K., Kinoshita, K., Uchino, H. & Hinuma, Y. 1984. Specific cytolysis of fresh tumor cells by an autologous killer T cell line derived from an adult T cell leukemia/lymphoma patient. *Journal of Immunology*, 133, 2, 1037-1041.

Kannagi, M., Harada, S., Maruyama, I. Inoko, H., Igarashi, H., Kuwashima, G., Sato, S., Morita, M., Kidokoro, M., Sugimoto, M., Funahashi, S., Osame, M. & Shida, H. 1991. Predominant recognition of human T cell leukemia virus type I (HTLV-I) pX gene products by human CD8+ cytotoxic T cells directed against HTLV- I-infected cells. *International Immunology*, 3, 8, 761-7

Kannagi, M. Immunologic control of human T-cell leukemia virus type I and adult T-cell leukemia. 2007. International Journal of Hematology, 86, 2, 113-117

Kannagi, M., Hasegawa, A., Kinpara, S., Shimizu, Y., Takamori, A. & Utsunomiya, A. 2011. Double control systems for human T-cell leukemia virus type 1 by innate and acquired immunity. *Cancer Science*, 102, 4, 670-6

Karube, K., Ohshima, K., Tsuchiya, T., Yamaguchi, T., Kawano, R., Suzumiya, J., Utsunomiya, A., Harada, M. & Kikuchi, M. 2004. Expression of FoxP3, a key molecule in CD4CD25 regulatory T cells, in adult T-cell leukaemia/lymphoma cells. *British Journal of Haematology*, 126, 1, 81-84

Kim, C.H., Butcher, E.C. & Johnston, B. 2002. Distinct subsets of human Valpha24-invariant NKT cells: cytokine responses and chemokine receptor expression. *Trends in Immunology*, 23, 11, 516-519

Kohno, T., Yamada, Y., Akamatsu, N., Kamihira, S., Imaizumi, Y., Tomonaga, M. & Matsuyama, T. 2005. Possible origin of adult T-cell leukemia/lymphoma cells from human T lymphotropic virus type-1-infected regulatory T cells. *Cancer Science, 96*, 8, 527-533

Kozako, T., Arima, N., Toji, S., Masamoto, I., Akimoto, M., Hamada, H., Che, X.F., Fujiwara, H., Matsushita, K., Tokunaga, M., Haraguchi, K., Uozumi, K., Suzuki, S., Takezaki, T. & Sonoda, S. 2006. Reduced frequency, diversity, and function of human T cell leukemia virus type 1-specific CD8+ T cell in adult T cell leukemia patients. *Journal of Immunology*, 177, 8, 5718-5726

Kubota, R., Fujiyoshi, T., Izumo, S., Yashiki, S., Maruyama, I., Osame, M. & Sonoda, S. 1993. Fluctuation of HTLV-I proviral DNA in peripheral blood mononuclear cells of HTLV-I-associated myelopathy. *Journal of Neuroimmunology*, 42, 2, 147-154

Kubota, R., Nagai, M., Kawanishi, T., Osame, M. & Jacobson, S. 2000. Increased HTLV type 1 tax specific CD8+ cells in HTLV type 1-associated myelopathy/tropical spastic paraparesis: correlation with HTLV type 1 proviral load. *AIDS Research and Human Retroviruses*, 16, 16, 1705-1709

Kubota, R., Soldan, S.S., Martin, R. & Jacobson, S. 2002. Selected cytotoxic T lymphocytes with high specificity for HTLV-I in cerebrospinal fluid from a HAM/TSP patient. Journal of Neurovirology, 8, 1, 53–57

Levin, M.C., Lehky, T.J., Flerlage, A.N., Katz, D., Kingma, D.W., Jaffe, E.S., Heiss, J.D., Patronas, N., McFarland, H.F. & Jacobson, S. 1997. Immunologic analysis of a spinal cord-biopsy specimen from a patient with human T-cell lymphotropic virus type I-associated neurologic disease. *New England Journal of Medicine*, 336, 12, 839–845

Macatonia, S.E., Cruickshank, J.K., Rudge, P. & Knight, S.C. 1992. Dendritic cells from patients with tropical spastic paraparesis are infected with HTLV-1 and stimulate autologous lymphocyte proliferation. *AIDS Research and Human Retroviruses*, 8, 9, 1699-1706

Macnamara, A., Rowan, A., Hilburn, S., Kadolsky, U., Fujiwara, H., Suemori, K., Yasukawa, M., Taylor, G., Bangham, C.R. & Asquith, B. 2010. HLA class I binding of HBZ determines outcome in HTLV-1 infection. *PLoS Pathogens*, 6, 9, e1001117

Matsubar, Y., Hori, T., Morita, R., Sakaguchi, S. & Uchiyama, T. 2006. Delineation of immunoregulatory properties of adult T-cell leukemia cells. *International Journal of Hematology*, 84, 1, 63–69

Matsuura, E., Yamano, Y. & Jacobson, S. 2010. Neuroimmunity of HTLV-I Infection. *Journal of Neuroimmune Pharmacology*, 5, 3, 310-25

Michaëlsson, J., Barbosa, H.M., Jordan, K.A., Chapman, J.M., Brunialti, M.K., Neto, W.K., Nukui, Y., Sabino, E.C., Chieia, M.A., Oliveira, A.S.B., Nixon, D.F. & Kallas, E.G. 2008. The frequency of CD127low expressing CD4+CD25high T regulatory cells is inversely correlated with human T lymphotrophic virus type-1 (HTLV-1) proviral load in HTLV-1-infection and HTLV-1-associated myelopathy/tropical spastic paraparesis. *BMC Immunology*, 9, 41

Moll, M., Kuylenstierna, C., Gonzalez, V.D., Andersson, S.K., Bosnjak, L., Sönnerborg, A., Quigley, M.F. & Sandberg, J.K. 2009.Severe functional impairment and elevated PD-1 expression in CD1d-restricted NKT cells retained during chronic HIV-1 infection. *European Journal of Immunology*, 39, 3, 902-911

Mosley, A.J., Asquith, B. & Bangham, C.R. 2005. Cell-mediated immune response to human T-lymphotropic virus type I. *Viral Immunology*, 18, 2, 293-305

Nagai, M., Usuku, K., Matsumoto, W., Kodama, D., Takenouchi, N., Moritoyo, T., Hashiguchi, S., Ichinose, M., Bangham, C.R., Izumo, S. & Osame, M. 1998. Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. *Journal of Neurovirology*, 4, 6, 586-593

Nagai, M., Kubota, R., Greten, T.F., Schneck, J.P., Leist, T.P. & Jacobson, S. 2001a. Increased activated human T cell lymphotropic virus type I (HTLV-I) Tax11-19-specific memory and effector CD8+ cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis: correlation with HTLV-I provirus load. *Journal of Infectious Diseases*, 183, 2, 197–205

Nagai, M., Yamano, Y., Brennan, M.B., Mora, C.A. & Jacobson, S. 2001b. Increased HTLV-I proviral load and preferential expansion of HTLV-I Tax-specific CD8+ T cells in cerebrospinal fluid from patients with HAM/TSP. *Annals of Neurology*, 50, 6, 807–812

Oh, U., Grant, C., Griffith, C., Fugo, K., Takenouchi, N. & Jacobson, S. 2006. Reduced Foxp3 protein expression is associated with inflammatory disease during human t lymphotropic virus type 1 Infection. *Journal of Infectious Diseases*, 193, 11, 1557–1566

Ohsugi, T. & Kumasaka, 2011. T. Low CD4/CD8 T-cell ratio associated with inflammatory arthropathy in human T-cell leukemia virus type I Tax transgenic mice. *PLoS One*, 6, 4, e18518

Osame, M., Usuku, K., Izumo, S., Ijichi, N., Amitani, H., Igata, A., Matsumoto, M. & Tara, M. 1986. HTLV-I associated myelopathy, a new clinical entity. *Lancet* 1, 8488, 1031–1032

Parker, C.E., Daenke, S., Nightingale, S. & Bangham, C.R. 1992. Activated, HTLV-1-specific cytotoxic T-lymphocytes are found in healthy seropositives as well as in patients with tropical spastic paraparesis. *Virology*. 188, 2, 628-636

Pique, C., Ureta-Vidal, A., Gessain, A., Chancerel, B., Gout, O., Tamouza, R., Agis, F. & Dokhélar, M.C. Evidence for the chronic in vivo production of human T cell leukemia virus type I Rof and Tof proteins from cytotoxic T lymphocytes directed against viral peptides. 2000. *Journal of Experimental Medicine*, 191, 3, 567–72

Ramirez, J.M., Brembilla, N.C., Sorg, O., Chicheportiche, R., Matthes, T., Dayer, J.M., Saurat, J.H., Roosnek, E., & Chizzolini, C. 2010. Activation of the aryl hydrocarbon receptor reveals distinct requirements for IL-22 and IL-17 production by human T helper cells. *European Journal of Immunology*, 40, 9, 2450–2459

Richardson, J.H., Edwards, A.J., Cruickshank, J.K., Rudge, P. & Dalgleish, A.G. In vivo cellular tropism of human T-cell leukemia virus type 1. 1990. *Journal of Virology*, 64, 11, 5682–5687

Roncador, G., Garcia, J.F., Maestre, L., Lucas, E., Menarguez, J., Ohshima, K., Nakamura, S., Banham, A.H., Piris, M.A. FOXP3, a selective marker for a subset of adult T-cell leukaemia/lymphoma. *Leukemia*, 19, 12, 2247–2253

Sabouri, A.H., Usuku, K., Hayashi, D., Izumo, S., Ohara, Y., Osame, M. & Saito, M. 2008 Impaired function of human T-lymphotropic virus type 1 (HTLV-1)-specific CD8+ T cells in HTLV-1-associated neurologic disease. *Blood*, 112, 6, 2411–2420

Saito, M., Braud, V.M., Goon, P., Hanon, E., Taylor, G.P., Saito, A., Eiraku, N., Tanaka, Y., Usuku, K., Weber, J.N., Osame, M. & Bangham, C.R. 2003. Low frequency of CD94/NKG2A+ T lymphocytes in patients with HTLV-1-associated myelopathy/tropical spastic paraparesis, but not in asymptomatic carriers. *Blood*, 102, 2, 577-584

Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M. & Toda, M. 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *Journal of Immunology*, 155, 3, 1151–1164

Sakaguchi, S., Yamaguchi, T., Nomura, T., & Ono, M. 2008. Regulatory T cells and immune tolerance. *Cell*, 133, 5, 775–787

Sandberg, J.K., Fast, N.M., Palacios, E.H., Fennelly, G., Dobroszycki, J., Palumbo, P., Wiznia, A., Grant, R.M., Bhardwaj, N., Rosenberg, M.G. & Nixon, D.F. 2002. Selective loss of innate CD4(+) V alpha 24 natural killer T cells in human immunodeficiency virus infection. *Journal of Virology*, 76, 15, 7528-7534

Sato, T., Araya, N. & Yamano, Y. 2011. Human T-lymphotropic virus type 1 (HTLV-1) and innate immunity. *Inflammation and Regeneration*, 31, 1, 110-115

Satou, Y & Matsuoka, M. 2010. HTLV-1 and the host immune system: how the virus disrupts immune regulation, leading to HTLV-1 associated diseases. *Journal of Clinical and Experimental Hematopathology*. 50, 1, 1-8

Satou, Y., Yasunaga, J., Zhao, T., Yoshida, M., Miyazato, P., Takai, K., Shimizu, K., Ohshima, K., Green, P.L., Ohkura, N., Yamaguchi, T., Ono, M., Sakaguchi, S. & Matsuoka, M. 2011. HTLV-1 bZIP factor induces T-cell lymphoma and systemic inflammation in vivo. *PLoS Pathogens*, 7, 2, e1001274

Shimauchi, T., Kabashima, K. & Tokura, Y. 2008. Adult T-cell leukemia/lymphoma cells from blood and skin tumors express cytotoxic T lymphocyte-associated antigen-4 and Foxp3 but lack suppressor activity toward autologous CD8+ T cells. *Cancer Science*, 99, 1, 98–106

Shimizu, Y., Takamori, A., Utsunomiya, A., Kurimura, M., Yamano, Y., Hishizawa, M., Hasegawa, A., Kondo, F., Kurihara, K., Harashima, N., Watanabe, T., Okamura, J., Masuda, T. & Kannagi, M. 2009. Impaired Tax-specific T-cell responses with insufficient control of HTLV-1 in a subgroup of individuals at asymptomatic and smoldering stages. *Cancer Science*, 100, 3, 481–489

Toulza, F., Heaps, A., Tanaka, Y., Taylor, G.P. & Bangham, C.R. 2008. High frequency of CD4+FoxP3+ cells in HTLV-1 infection: inverse correlation with HTLV-1-specific CTL response. *Blood* 111, 10, 5047–5053

Tsuji, M., Komatsu, N., Kawamoto, S., Suzuki, K., Kanagawa, O., Honjo, T., Hori, S. & Fagarasan, S. 2009. Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. *Science* 323, 5920, 1488–1492

Uchiyama, T., Yodoi, J., Sagawa, K., Takatsuki, K. & Uchino, H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. 1977. *Blood*, 50, 3, 481-92

Umehara, F., Izumo, S., Nakagawa, M., Ronquillo, A.T., Takahashi, K., Matsumuro, K., Sato, E. & Osame M. 1993. Immunocytochemical analysis of the cellular infiltrate in the spinal cord lesions in HTLV-Iassociated myelopathy. *Journal of Neuropathology and Experimental Neurology*, 52, 4, 424–430

Umehara, F., Nakamura, A., Izumo, S., Kubota, R., Ijichi, S., Kashio, N., Hashimoto, K., Usuku, K., Sato, E. & Osame, M. 1994. Apoptosis of T lymphocytes in the spinal cord lesions in HTLV-I-associated myelopathy: a possible mechanism to control viral infection in the central nervous system. *Journal of Neuropathology and Experimental Neurology*, 53, 6, 617–624

van der Vliet, H.J., von Blomberg, B.M., Hazenberg, M.D., Nishi, N., Otto, S.A., van Benthem, B.H., Prins, M., Claessen, F.A., van den Eertwegh, A.J., Giaccone, G., Miedema, F., Scheper, R.J. & Pinedo, H.M. 2002. Selective decrease in circulating V alpha 24+V beta 11+ NKT cells during HIV type 1 infection. *Journal of Immunology*, 168, 3, 1490-1495

Wodarz, D., Nowak, M.A. & Bangham, C.R. 1999. The dynamics of HTLV-I and the CTL response. *Immunology Today*, 20, 5, 220–227

Wodarz, D., Hall, S.E., Usuku, K., Osame, M., Ogg, G.S., McMichael, A.J., Nowak, M.A. & Bangham, C.R.M.. 2001. Cytotoxic T-cell abundance and virus load in human immunodeficiency virus type 1 and human T-cell leukaemia virus type 1. Proceedings of the Royal Society of London B, 268, 1473, 1215–21Yamano, Y., Nagai, M., Brennan, M. Mora, C.A., Soldan, S.S., Tomaru, U., Takenouchi, N., Izumo, S., Osame, M. & Jacobson, S. 2002. Correlation of human T-cell

lymphotropic virus type 1 (HTLV-1) mRNA with proviral DNA load, virusspecific CD8(+) T cells, and disease severity in HTLV-1-associated myelopathy (HAM/TSP). *Blood* 99, 1, 88–94

Yamano, Y., Takenouchi, N., Li, H.C., Tomaru, U., Yao, K., Grant, C.W., Maric, D.A. & Jacobson, S. 2005. Virus-induced dysfunction of CD4+CD25+ T cells in patients with HTLV-I-associated neuroimmunological disease. *Journal of Clinical Investigations*, 115, 5, 1361–1368

Yamano, Y., Araya, N., Sato, T., Utsunomiya, A., Azakami, K., Hasegawa, D., Izumi, T., Fujita, H., Aratani, S., Yagishita, N., Fujii, R., Nishioka, K., Jacobson, S. & Nakajima, T. 2009. Abnormally high levels of virus-infected IFN-gamma+ CCR4+ CD4+ CD25+ T cells in a retrovirus-associated neuroinflammatory disorder. *PLoS One, 4*, 8, e6517

Yu, F., Itoyama, Y., Fujihara, K. & Goto, I. 1991. Natural killer (NK) cells in HTLV-I-associated myelopathy/tropical spastic paraparesis-decrease in NK cell subset populations and activity in HTLV-I seropositive individuals. Journal of Neuroimmunology, 33, 2, 121-128

Yoshie, O., Imai, T. & Nomiyama, H. 2001. Chemokines in immunity. *Advances in Immunology*, 78, 57–110

Yoshie, O., Fujisawa, R., Nakayama, T., Harasawa, H., Tago, H., Izawa, D., Hieshima, K., Tatsumi, Y., Matsushima, K., Hasegawa, H., Kanamaru, A., Kamihira, S. & Yamada, Y. 2002. Frequent expression of CCR4 in adult T-cell leukemia and human T-cell leukemia virus type 1-transformed T cells. *Blood*, 99, 5, 1505–11

Zhou, X., Bailey-Bucktrout, S.L., Jeker, L.T., Penaranda, C., Martinez-Llordella, M., Ashby, M., Nakayama, M., Rosenthal, W. & Bluestone, J.A. 2009. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nature Immunology*, 10, 9, 1000–1007

Zhu, J. & Paul, W.E. 2010. Heterogeneity and plasticity of T helper cells. Cell Research, 20, 1, 4-12

Viruses **2011**, *3*, 1532-1548; doi:10.3390/v3091532

OPEN ACCESS

viruses

ISSN 1999-4915

www.mdpi.com/journal/viruses

Review

Human T-Lymphotropic Virus Type 1 (HTLV-1) and Regulatory T Cells in HTLV-1-Associated Neuroinflammatory Disease

Natsumi Araya [1], Tomoo Sato [1], Naoko Yagishita [1], Hitoshi Ando [1], Atae Utsunomiya [2], Steven Jacobson [3] and Yoshihisa Yamano [1,*]

[1] Department of Rare Diseases Research, Institute of Medical Science, School of Medicine, St. Marianna University, Kawasaki 216-8511, Japan; E-Mails: araya@marianna-u.ac.jp (N.A.); tomoo@marianna-u.ac.jp (T.S.); yagi@marianna-u.ac.jp (N.Y.); hando@marianna-u.ac.jp (H.A.)

[2] Department of Hematology, Imamura Bun-in Hospital, Kagoshima 890-0064, Japan; E-Mail: autsunomiya@jiaikai.jp

[3] Viral Immunology Section, Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA; E-Mail: jacobsons@ninds.nih.gov

* Author to whom correspondence should be addressed; E-Mail: yyamano@marianna-u.ac.jp; Tel.: +81-44-977-8111; Fax: +81-44-977-9772.

Received: 2 June 2011; in revised form: 13 August 2011 / Accepted: 16 August 2011 / Published: 25 August 2011

Abstract: Human T-lymphotropic virus type 1 (HTLV-1) is a retrovirus that is the causative agent of adult T cell leukemia/lymphoma (ATL) and associated with multiorgan inflammatory disorders, including HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and uveitis. HTLV-1-infected T cells have been hypothesized to contribute to the development of these disorders, although the precise mechanisms are not well understood. HTLV-1 primarily infects $CD4^+$ T helper (Th) cells that play a central role in adaptive immune responses. Based on their functions, patterns of cytokine secretion, and expression of specific transcription factors and chemokine receptors, Th cells that are differentiated from naïve $CD4^+$ T cells are classified into four major lineages: Th1, Th2, Th17, and T regulatory (Treg) cells. The $CD4^+CD25^+CCR4^+$ T cell population, which consists primarily of suppressive T cell subsets, such as the Treg and Th2 subsets in healthy individuals, is the predominant viral reservoir of HTLV-1 in both ATL and HAM/TSP patients. Interestingly, $CD4^+CD25^+CCR4^+$ T cells become Th1-like cells in

HAM/TSP patients, as evidenced by their overproduction of IFN-γ, suggesting that HTLV-1 may intracellularly induce T cell plasticity from Treg to IFN-γ^+ T cells. This review examines the recent research into the association between HTLV-1 and Treg cells that has greatly enhanced understanding of the pathogenic mechanisms underlying immune dysregulation in HTLV-1-associated neuroinflammatory disease.

Keywords: HTLV-1; HAM/TSP; ATL; $CD4^+CD25^+CCR4^+$ T cell; regulatory T cell; ex$Foxp3^+$ cell; inflammation; immune-dysfunction

1. Introduction

Human T-lymphotropic virus type 1 (HTLV-1) is a retrovirus associated with chronic, persistent infection of human T cells. HTLV-1 infection is endemic in Japan, the Caribbean, and part of South America, Africa, the Middle East, and Melanesia [1]. Studies conducted in HTLV-1 endemic areas have demonstrated that HTLV-1 infection is associated with a variety of human diseases, including an aggressive mature T cell malignancy termed adult T-cell leukemia (ATL) [2], which is defined as neoplastic growth of HTLV-1-infected T cells. HTLV-1 is also associated with non-neoplastic inflammatory conditions such as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [3,4], uveitis [5], Sjögren syndrome [6], bronchoalveolitis, arthritis [7], and polymyositis [8], where high tissue concentrations of HTLV-1 infected T lymphocytes have been observed. Importantly, some patients have more than one of these HTLV-1-associated inflammatory conditions [9].

Although HTLV-1-associated disorders have been extensively studied, the exact mechanism by which HTLV-1 induces these inflammatory conditions is not completely understood. The proviral load of HTLV-1 may contribute to development of HTLV-1-associated inflammatory conditions, since the number of HTLV-1-infected T cells circulating in the peripheral blood is higher in patients with HAM/TSP than in asymptomatic HTLV-1-infected individuals [10,11], and is even higher in the cerebrospinal fluid of patients with HAM/TSP [12]. In HAM/TSP patients, the proviral load correlates with not only the percentage of activated $CD4^+$ T cells but also with that of HTLV-1-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) [11,13]. These HTLV-1-specific CTLs produce various cytokines, such as IFN-γ and TNF-α, that may suppress viral replication and kill infected cells and/or promote bystander activation and killing of nearby resident cells in the central nervous system (CNS) [14–17]. In addition, increased viral expression, particularly of the transactivating viral gene encoding HTLV-1 Tax, has also been hypothesized to play a role in HTLV-1 disease progression [11,12]. Transgenic mice expressing HTLV-1 Tax develop an inflammatory arthropathy [18], and transgenic rats expressing HTLV-1 env-pX develop destructive arthropathy, Sjögren syndrome, vasculitis, and polymyositis [19]. These findings support the hypothesis that HTLV-1 *tax* is one of the exogenous retrovirus genes responsible for immune dysregulation.

HTLV-1 Tax is a transactivator/oncoprotein that has potent effects on infected T cells, including activation of nuclear factor(NF)-κB [20] with subsequent enhancement of cell activation and proliferation and expression of various cellular genes, such as IL-2 [21], the α-chain of the IL-2 receptor (IL-2Rα) [22], IL-15 [23], and IL-15Rα [24]. Such virus-induced intracellular activation may

directly contributes to T cell activation and the *ex vivo* T cell proliferation observed in patients with HAM/TSP [25]. These findings suggest that invasion by HTLV-1-infected T cells, together with viral gene expression and cellular-signaling mechanisms, trigger a strong virus-specific immune response and increased proinflammatory cytokine production, leading to CNS inflammation and autologous tissue damage. However, the precise mechanisms underlying the induction of immune activation by HTLV-1-infected T cells are not well understood.

2. HTLV-1 and Regulatory T Cells

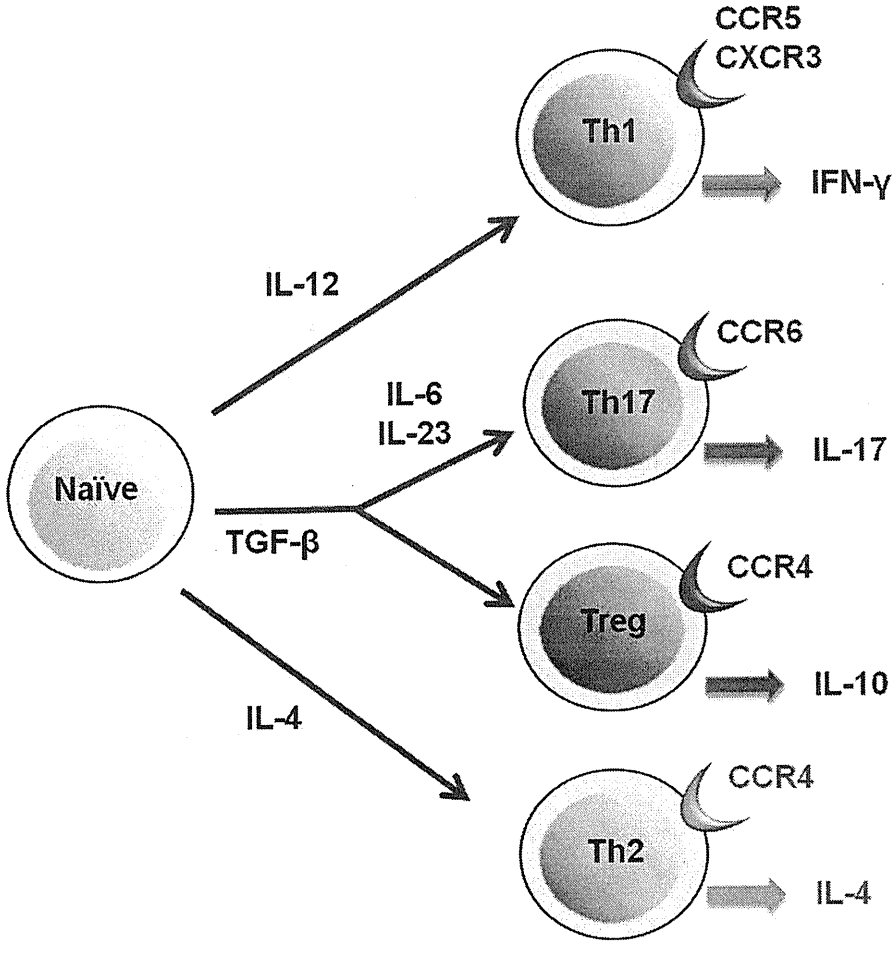
The recent discovery of regulatory T cells (Treg cells) has generated new opportunities for and increased interest in elucidating the above mentioned mechanisms. In healthy individuals, the Treg cells, a subset of $CD4^+CD25^+$ T cells, play a key role in maintaining immune system homeostasis by suppressing the proliferation of and cytokine production by pathogenic T cells [26]. Although Treg cells are phenotypically similar to activated T cells, they can be identified *ex vivo* by their intracellular expression of the transcriptional regulator Foxp3 [27], which is critical in the development and functioning of Treg cells in both mice and humans. Significant reductions in Foxp3 expression and/or Treg cell function have been observed in patients with several types of human autoimmune diseases [28], suggesting that defects in Foxp3 expression and/or Treg functioning may precipitate loss of immunological tolerance. $CD4^+CD25^+$ T cells are also the predominant viral reservoir in the peripheral blood of HTLV-1-infected individuals [29]. Recently, significant reductions in Foxp3 expression and Treg cell function have been observed in $CD4^+CD25^+$ T cells from patients with HAM/TSP [30–34]. Furthermore, decreased expression levels of CTL antigen-4 (CTLA-4), a Treg-associated immune-suppressive molecule, and glucocorticoid-induced tumor necrosis factor receptor-related protein (GITR) have also been observed on the $CD4^+CD25^+$ T cells of HAM/TSP patients [30,34]. Notably, overexpression of HTLV-1 Tax has been observed to reduce Foxp3 expression and inhibit the suppressive function of Treg cells *in vitro* [30]. Furthermore, because of a Tax-induced defect in TGF-β signaling, Foxp3 expression was decreased and Treg functions were impaired in patients with HAM/TSP [35]. Recently, significantly decreased numbers of $CD4^+CD25^+Foxp3^+$ Treg cells were observed in transgenic mice expressing HTLV-1 Tax that develop an inflammatory arthropathy [36]. In addition, increased viral expression of the HTLV-1 bZIP factor (*HBZ*) gene encoding the minus strand of HTLV-1 has also been suggested to play a role in HTLV-1 disease progression [37], and $CD4^+Foxp3^+$ Treg cells in HBZ transgenic mice were functionally impaired [38]. These findings indicate that HTLV-1-induced dysfunctioning of $CD4^+CD25^+$ Treg cells may be one of the mechanisms underlying the induction of immune activation by HTLV-1-infected T cells.

In contrast to the decreased expression of Foxp3 in $CD4^+CD25^+$ T cells observed in HAM/TSP patients [30–34], most $CD4^+CD25^+$ ATL cells have been shown to express Foxp3 in patients with ATL [39,40]. Therefore, it has been hypothesized that ATL cells may be derived from Treg cells [41]. Interestingly, some ATL cells exhibit immunosuppressive functions similar to those of Treg cells, which may contribute to clinically observed cellular immunodeficiency in ATL patients [41–43], although some of these ATL cells lose this regulatory function [44].

3. HTLV-1 and $CD4^+CD25^+CCR4^+$ T Cells

Although HTLV-1 has been reported to infect a number of cell types both *in vitro* and *in vivo* [29,45–49], $CD4^+$ Th cells, which play a central role in adaptive immune responses, are the predominant viral reservoir in the peripheral blood [50]. To understand the effects of HTLV-1 infection on the functioning of $CD4^+$ Th cells, it is necessary to discover if, and if so which of the Th subpopulations is preferentially infected with HTLV-1. Based on their functions, patterns of cytokine secretion, and expression of specific transcription factors and chemokine receptors, $CD4^+$ Th cells, which are differentiated from naïve $CD4^+$ T cells, are classified into four major lineages: Th1, Th2, Th17, and Treg cells (Figure 1).

Figure 1. T cell subsets of $CD4^+$ T helper cells. Th cells are differentiated from naïve $CD4^+$ T cells into 4 major lineages: Th1, Th2, Th17, and T-regulatory (Treg) cells. Each Th subset exhibits characteristic functions, patterns of cytokine secretion, and expression of specific chemokine receptors.



The chemokine receptor CCR4 has recently been found to be expressed on HTLV-1-infected leukemia cells in ATL patients [51]. Because CCR4 is known to be selectively expressed on Treg and Th2 cells [51–53] (Figure 1) and because most ATL cells express high levels of Foxp3, it has been hypothesized that ATL cells may be derived from Treg cells [41]. Although it has been

demonstrated that $CD4^+CD25^+$ T cells in HAM/TSP patients exhibit reduced Foxp3 expression and Treg suppression [30–33] and that HTLV-1-infected $CD4^+$ T cells in HAM/TSP patients produce Th1 cytokines (IFN-γ) [16,30], it has also been observed that CCR4 selectively overexpresses on HTLV-1-infected T cells in HAM/TSP patients [54]. Furthermore, the majority of $CD4^+CD25^+CCR4^+$ T cells have been found to be infected with HTLV-1 and this T cell subset has increased numbers in HAM/TSP patients [54]. Thus, $CD4^+CD25^+CCR4^+$ T cells are a major reservoir of HTLV-1-infected T cells, which are increased in numbers in both HAM/TSP and ATL patients.

4. HTLV-1 and $Foxp3^-CD4^+CD25^+CCR4^+$ T Cells

Although CCR4 is known to be selectively expressed on Treg and Th2 cells in healthy individuals, more detailed flow cytometric analysis of Foxp3 expression in $CD4^+CD25^+CCR4^+$ T cells of HAM/TSP patients demonstrated that the frequency of the $Foxp3^-$ population was greatly increased in $CD4^+CD25^+CCR4^+$ T cells [54]. Moreover, analysis of proinflammatory cytokine expression in this $Foxp3^-CD4^+CD25^+CCR4^+$ T cell subset demonstrated that these cells uniquely produced multiple proinflammatory cytokines such as IL-2, IL-17, and few IFN-γ in healthy individuals while $Foxp3^+CD4^+CD25^+CCR4^+$ T cells (Treg cells) did not. Furthermore, it was demonstrated that HAM/TSP patients had only few $Foxp3^+CD4^+CD25^+CCR4^+$ T cells that did not produce such cytokines [54]. The $Foxp3^-CD4^+CD25^+CCR4^+$ T cells in HAM/TSP were greater in number and overproduced IFN-γ [54]. Further, the proportion of these IFN-γ-producing $Foxp3^-CD4^+CD25^+CCR4^+$ T cells may have a functional consequence, since the presence of this subpopulation could be correlated with disease activity and severity of HAM/TSP *in vivo* [54]. Thus, in a $CD4^+CD25^+CCR4^+$ T cell population that mainly consists of suppressive T cell subsets such as Treg and Th2 under healthy conditions, IFN-γ-producing $Foxp3^-CD4^+CD25^+CCR4^+$ T cells, rarely encountered in healthy individuals, were increased in number and overproduced IFN-γ in HAM/TSP patients (Figure 2). We therefore propose to call this IFN-$\gamma^+$$Foxp3^-CD4^+CD25^+CCR4^+$ T cell subset T_{HAM} cells. Interestingly, increased numbers of $Foxp3^{low}CD4^+CD25^+$ memory T cells, which have cytokine secretion patterns similar to those of T_{HAM} cells, have recently been observed in patients with active systemic lupus erythematosus (SLE) [55]. Therefore, it would be of interest to build on this finding by confirming whether this newly defined unique T cell subset, which has been observed in both HAM/TSP and SLE patients, is found in both these patient groups and can be functionally deregulated in other immunological diseases.

Although most $CD4^+CD25^+CCR4^+$ T cells are infected with HTLV-1 in both HAM/TSP and ATL patients [54,56], the ratio of T_{HAM} cells ($CCR4^+Foxp3^-$ with IFN-γ production) to Treg cells ($CCR4^+Foxp3^+$ with no cytokine production) in the $CD4^+CD25^+CCR4^+$ T cell subset has been found to be high in HAM/TSP patients but low in ATL patients [54]. This differential T_{HAM}/Treg ratio in HTLV-1-infected T cells may be associated with the differential immune responses observed between HAM/TSP and ATL patients (Figure 3). ATL patients tend to have very low numbers of Tax-specific $CD8^+$ T cells in peripheral blood mononuclear cells (PBMCs) and to develop opportunistic infections [57,58], while HAM/TSP patients tend to have high numbers of Tax-specific $CD8^+$ CTLs [11,12,14,59]. As $CD4^+CD25^+$ T cells with high levels of Foxp3 expression have been reported to have an immunosuppressive function in ATL patients [41–43], the increased number of

$CD4^+CD25^+CCR4^+$ leukemia T cells with Treg functions observed in ATL patients may contribute to their clinically observed cellular immunodeficiency. However, HAM/TSP patients show very high cellular and humoral immune responses, such as high proportions of Tax-specific $CD8^+$ T cells, as well as cytomegalovirus (CMV)-specific $CD8^+$ T cells in the PBMCs [14,33]; high antibody titer to HTLV-1 [9]; and increased production of proinflammatory cytokines, such as IL-12 and IFN-γ [60]. It has been reported that $CD4^+CD25^+$ T cells with low expression of Foxp3 [30] and HTLV-1 Tax-expressing $Foxp3^+$ Treg cells [61] extracted from HAM/TSP patients exhibit defective immunosuppressive functioning. Moreover, it has been demonstrated that HTLV-1-infected IFN-γ-overproducing $CD4^+CD25^+CCR4^+Foxp3^-$ T cells (T_{HAM} cells) increase in number in HAM/TSP patients, and their levels can be correlated with disease severity [54]. Thus, $CD4^+CD25^+CCR4^+$ T cells with increased proinflammatory functioning, together with a defective Treg compartment [30–33,54], may overcome the regulatory effect of HTLV-1-uninfected Treg cells [61] and at least partly account for the heightened immune response observed in HAM/TSP patients. Collectively, these observations support the hypothesis that an imbalance in the T_{HAM}/Treg ratio in HTLV-1-infected $CD4^+CD25^+CCR4^+$ T cells is an important contributing factor in the immunological differences in host immune response observed between HAM/TSP and ATL patients (Figure 3).

Figure 2. Cellular components of $CD4^+CD25^+CCR4^+$ T cells in healthy donors and HAM/TSP patients. In healthy donors, the $CD4^+CD25^+CCR4^+$ T cell population primarily consists of suppressive T cell subsets, such as Treg and Th2, whereas that of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients consists of an increased number of IFN-γ-producing $Foxp3^-CD4^+CD25^+CCR4^+$ T cells (T_{HAM} cells).

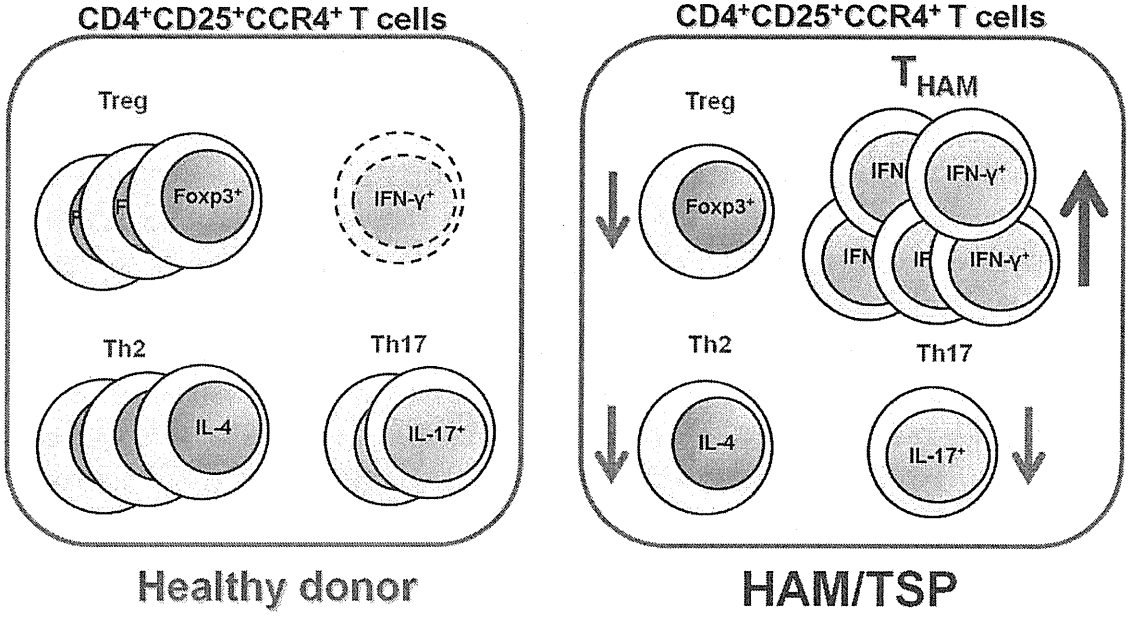
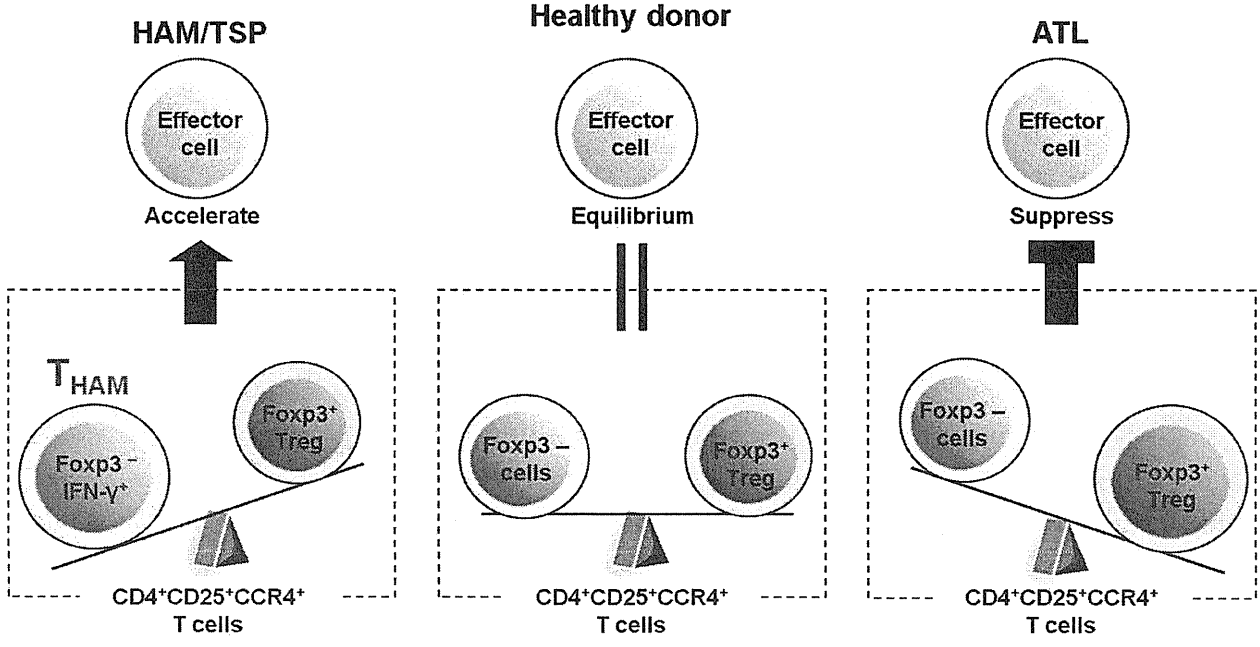


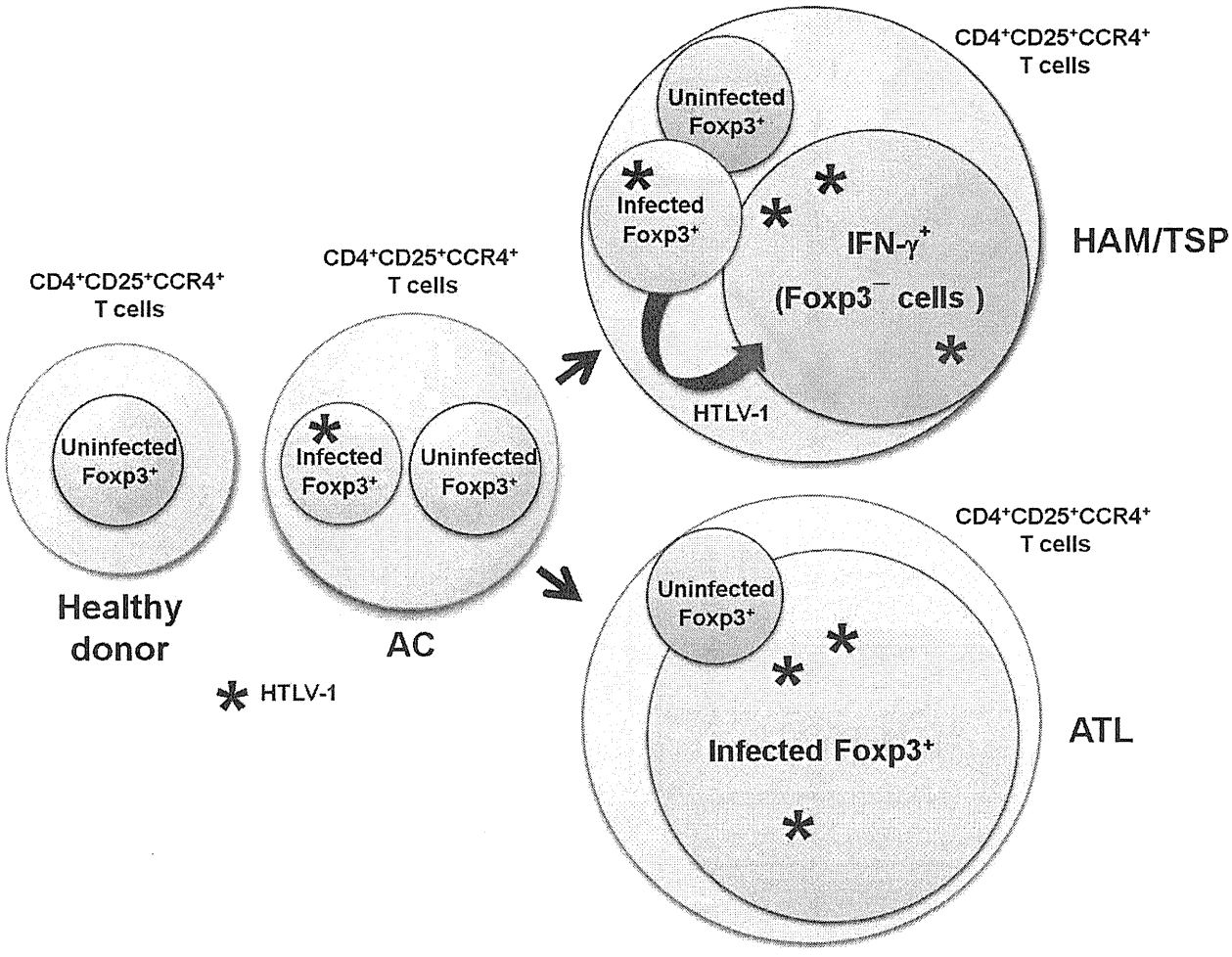
Figure 3. Differential immune responses and T_{HAM}/Treg ratios in $CD4^+CD25^+CCR4^+$ T cells in HAM/TSP and adult T cell leukemia/lymphoma (ATL) patients.



5. Increased Numbers of $CD4^+Foxp3^+$ Cells in HAM/TSP Patients

Recently, it has been reported that the number of $CD4^+Foxp3^+$ cells increases in HTLV-1-infected asymptomatic carriers, and is even higher in patients with HAM/TSP [61]. Although this report initially appears to conflict with the observations described above, it may not. In contrast to the decreased number of $CD4^+$ T cells in patients with human immunodeficiency virus (HIV) infection, the number of HTLV-1 infected $CD4^+$ T cells—most of which are CD4+CD25+CCR4+ T cells—in HAM/TSP patients is greatly increased. Therefore, although the percentage of $Foxp3^+$ cells among the $CD4^+CD25^+CCR4^+$ T cells is lower, the overall number of $CD4^+Foxp3^+$ cells in HAM/TSP patients may be higher than that in healthy donors (Figure 4). Indeed, when we analyzed the number of $Foxp3^+$ cells in healthy donors and HAM/TSP patients, we found it to be nearly equivalent between the two groups or slightly higher in HAM/TSP patients [54]. This difference (from slightly high to higher) would depend on the number of HTLV-1-infected $CD4^+$ T cells in the samples tested. Importantly, Toulza *et al.* demonstrated that the rate of CTL-mediated lysis was negatively correlated with the number of HTLV-1-$Tax^-CD4^+Foxp3^+$ cells, but not with the number of $Tax^+CD4^+Foxp3^+$ cells [61], again suggesting that HTLV-1-infected Treg cells lose their regulatory function, while HTLV-1-uninfected Treg cells contribute substantially to immune control of HTLV-1 infection.

Figure 4. Scheme of proportion of each cellular component in $CD4^+CD25^+CCR4^+$ T cells of healthy donors, asymptomatic carriers (AC), and patients with HAM/TSP or ATL. Although the proportion of $Foxp3^+$ cells among the $CD4^+CD25^+CCR4^+$ T cells is lower in HAM/TSP patients, the overall number of $CD4^+Foxp3^+$ cells in HAM/TSP patients is higher than that in healthy donors. In ATL patients, the majority of $CD4^+CD25^+CCR4^+$ T cells are $Foxp3^+$ cells.



6. Does the T_{HAM} Cell Population Include exFoxp3$^+$ Cells?

According to Hieshima *et al.*'s recent delineation of the molecular mechanism underlying HTLV-1 tropism to $CCR4^+CD4^+$ T cells [60], HTLV-1 Tax does not induce expression of CCR4, but Tax does induce expression of CCL22, which is the ligand for CCR4. Therefore, HTLV-1-infected T cells produce CCL22 through Tax and selectively interact with $CCR4^+CD4^+$ T cells, resulting in preferential transmission of HTLV-1 to $CCR4^+CD4^+$ T cells (Figure 5). In HTLV-1-seronegative healthy individuals, $CD4^+CD25^+CCR4^+$ T cell populations primarily consist of suppressive T cell subsets, such as Treg and Th2 cells [61]. However, as described above, cells of this T cell subset become Th1-like cells that overproduce IFN-γ in patients with HAM/TSP, while leukemogenesis develops and maintains the $Foxp3^+$ Treg phenotype in ATL patients (Figure 5).