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VI. 研究成果の刊行物・別刷

Host Immune System Abnormalities Among Patients with Human T-Lymphotropic Virus Type 1 (HTLV-1)-Associated Disorders

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1. Introduction

Human T-cell lymphotropic virus type 1 (HTLV-1) is a human retrovirus that causes persistent infection in the host. While most infected persons remain asymptomatic carriers (ACs), 3–5% develop a T-cell malignancy termed adult T-cell leukemia (ATL) (Uchiyama et al., 1977), and another 0.25–3% develop a chronic progressive inflammatory neurologic disease known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gessain et al., 1985; Osame et al. 1986). Although HTLV-1-associated disorders have been extensively studied, the exact mechanism by which they are induced by HTLV-1 is not completely understood. The proviral load of HTLV-1 could contribute to the development of these disorders, since the circulating number of HTLV-1-infected T cells in the peripheral blood is associated with the risk of developing HAM/TSP and ATL (Iwanaga et al., 2010; Nagai et al. 1998). However, more detail on the precise immune mechanisms controlling HTLV-1-infected cells is still needed.

HTLV-1 preferentially infects CD4⁺ T cells, the central regulators of the acquired immune system (Richardson et al., 1990). This is known to induce a variety of abnormalities, such as proliferation, cellular activation, and proinflammatory changes (Boxus et al., 2009; Satou et al., 2010; Yamano et al. 2009). These abnormalities, in turn, may deregulate the balance of the host immune system.

HTLV-1 also causes abnormalities among uninfected immune cells. Patients with HTLV-1-associated disorders demonstrate abnormalities in both the amount and function of CD8⁺ cytotoxic T lymphocytes (CTL), an important component of host immune response against HTLV-1 (Bangham 2009; Kannagi et al., 2011; Matsuura et al., 2010). Patients with ATL and HAM/TSP may also experience reductions in the amount and efficacy of cellular components of innate immunity, which is vital in regulating the immune response against general viral infections and cancers (Azakami et al., 2009; Matsuura et al., 2010). In this chapter, we have summarized the host immune system abnormalities that are associated with HTLV-1 infection.

2. Abnormality of HTLV-1-infected CD4⁺ T cells

2.1 CD4⁺CD25⁺CCR4⁺ T Cells are a major reservoir of HTLV-1-infected T cells, which increase in HAM/TSP and ATL patients

HTLV-1 mainly infects CD4⁺ T helper (Th) cells, which play a central role in adaptive immune responses (Richardson et al., 1990). CD4⁺ Th cells recruit and activate other immune cells, including B cells, CD8 T cells, macrophages, mast cells, neutrophils, eosinophils, and basophils (Zhu et al., 2010). Based on their function, their pattern of cytokine secretion, and their expression of specific transcription factors and chemokine receptors, CD4⁺ Th cells, differentiated from naïve CD4⁺ T cells, are classified into 4 major lineages: Th1, Th2, Th17, and T regulatory (Treg) cells. To understand the effects of HTLV-1 infection on the function of CD4 Th cells, it is necessary to know which Th population HTLV-1 infects.

It was recently shown that the chemokine receptor CCR4 is expressed on HTLV-1-infected leukemia cells in ATL patients (Yoshie et al., 2002). CCR4 is selectively expressed on suppressive T cell subsets, such as Treg and Th2 cells, in HTLV-1-seronegative healthy individuals (Yoshie et al., 2001). Using molecular and immunological techniques, we also demonstrated that CD4⁺CD25⁺CCR4⁺ T cells were the predominant viral reservoir in both ACs and HAM/TSP patients, and that this T cell subset was increased in HAM/TSP patients (Yamano et al., 2009). Thus, CD4⁺CD25⁺CCR4⁺ T cells are a major population of HTLV-1-infected T cells, which increase in number in both HAM/TSP and ATL patients.

The molecular mechanism of HTLV-1 tropism to CCR4 expressing CD4⁺ T cells was recently uncovered (Hieshima et al., 2008). HTLV-1 Tax, a transcriptional regulator encoded by the HTLV-1 genome, does not induce expression of CCR4, but it does induce expression of CCL22, the ligand for CCR4. Because HTLV-1-infected T cells selectively interact with CCR4⁺CD4⁺ T cells, this results in preferential transmission of HTLV-1 to CCR4⁺CD4⁺ T cells.

2.2 Differences in the fates of CD4⁺CD25⁺CCR4⁺ T cells in HAM/TSP and ATL patients

Among CD4⁺ Th cells, the major reservoir of HTLV-1 is CD4⁺CD25⁺CCR4⁺ T cells, including suppressive T cell subsets such as Treg and Th2 under healthy conditions. The exact mechanism by which HTLV-1 induces the deregulation of the host immune system is not completely understood. However, the recent discovery of Treg cells has provided new opportunities and generated increased interest in this issue. In healthy individuals, Treg cells suppress the proliferation of, and cytokine production by, pathogenic T cells, and thereby plays a key role in the maintenance of immune system homeostasis (Sakaguchi et al., 1995). Treg cells can be identified *ex vivo* by the intracellular expression of the transcriptional regulator Foxp3 (Hori et al., 2003), which is critical for the development and function of Treg cells in both mice and humans.

Significant reductions in Foxp3 expression and/or Treg cell function have been observed in several human autoimmune diseases (Sakaguchi et al., 2008), suggesting that defects in Foxp3 expression and/or Treg function may precipitate the loss of immunologic tolerance. Recently, significant reductions in Foxp3 expression and Treg cell function have also been observed in CD4⁺CD25⁺ T cells and/or CD4⁺CD25⁺CCR4⁺ T cells from patients with HAM/TSP (Hayashi et al., 2008; Michaelsson et al., 2008; Oh et al., 2006; Ramirez et al., 2010; Yamano et al., 2005). Furthermore, decreased expression levels of the Treg-associated immune suppressive molecules CTLA-4 and GITR were also observed on CD4⁺CD25⁺ T cells in HAM/TSP patients (Ramirez et al., 2010; Yamano et al., 2005). Notably, overexpression of HTLV-1 *tax* can reduce

Foxp3 expression and inhibit the suppressive function of Treg cells (Yamano et al., 2005). Furthermore, because of a Tax-induced defect in TGF- β signaling, HAM/TSP patients experience reductions in Foxp3 expression and impairment of Treg function (Grant et al., 2008). Moreover, a significant reduction in CD4⁺CD25⁺Foxp3⁺ Treg cells was demonstrated in HTLV-1-*tax*-expressing transgenic mice, which develop an inflammatory arthropathy (Ohsugi et al., 2011). Thus, HAM/TSP patients display a decreased ratio of Foxp3⁺ Treg cells within HTLV-1-infected CD4⁺CD25⁺CCR4⁺ T cells.

Importantly, a more detailed flow cytometric analysis of Foxp3 expression in CD4⁺CD25⁺CCR4⁺ T cells demonstrated that the frequency of "Foxp3⁻ population" was extraordinary high in HAM/TSP patients (Yamano et al., 2009). Moreover, an analysis of proinflammatory cytokine expression in this Foxp3⁻CD4⁺CD25⁺CCR4⁺ T cell subset demonstrated that these cells were unique because, in healthy individuals, they produced multiple proinflammatory cytokines such as IL-2, IL-17, and few interferon (IFN)- γ , while Foxp3⁺CD4⁺CD25⁺CCR4⁺ T cells (Treg cells) did not. Furthermore, HAM/TSP patients were found to exhibit only a few Foxp3⁺CD4⁺CD25⁺CCR4⁺ T cells that did not produce such cytokines. Rather, these patients had an increased number of Foxp3⁻CD4⁺CD25⁺CCR4⁺ T cells, which were found to overproduce IFN- γ . Further, given the increase of clinical diseases and severity of HAM/TSP observed in these patients, it appears likely that the frequency of these IFN- γ -producing Foxp3⁻CD4⁺CD25⁺CCR4⁺ T cells may have a functional consequence (Yamano et al., 2009). Thus, while the CD4⁺CD25⁺CCR4⁺ T cell population in healthy patients mainly comprises suppressive T cell subsets such as Treg and Th2, HAM/TSP patients possess an increased proportion of IFN- γ -producing Foxp3⁻CD4⁺CD25⁺CCR4⁺ T cells, which are rarely encountered in healthy individuals and lead to an overproduction of IFN- γ (Figure 1).

Although Foxp3 expression is decreased by CD4⁺CD25⁺ (CCR4⁺) T cells in HAM/TSP patients (Hayashi et al., 2008; Michaelsson et al., 2008; Oh et al., 2006; Ramirez et al., 2010; Yamano et al., 2005), it is increased by CD4⁺CD25⁺(CCR4⁺) ATL cells in most ATL patients (Karube et al., 2004; Roncador et al., 2005) (Figure 1). Therefore, it has been hypothesized that ATL cells may be derived from Treg cells (Kohno et al., 2005). Interestingly, some ATL cells exhibit immunosuppressive functions similar to those of Treg cells, which may contribute to the cellular immunodeficiency that has been clinically observed in ATL patients (Chen et al., 2006; Kohno et al., 2005; Matsubar et al., 2006); however, some ATL cells lose this regulatory function (Shimauchi et al., 2008).

2.3 HTLV-1 may induce plasticity of Foxp3⁺ cells into exFoxp3⁺ cell

In HTLV-1-seronegative healthy individuals, CD4⁺CD25⁺CCR4⁺ T cells mainly include suppressive T cell subsets such as Treg and Th2 (Yoshie et al., 2001). In ATL patients, most of this subset develops leukemogenesis by maintaining the Foxp3⁺ Treg phenotype (Figure 1). However, as mentioned above, T cells of this subset become Th1-like cells that overproduce IFN- γ in HAM/TSP patients (Figure 1). Since HTLV-1 may preferentially transmit to CCR4⁺CD4⁺ T cells, these findings suggest that HTLV-1 may intracellularly induce T-cell plasticity of Treg cells into IFN- γ ⁺ T cells. Indeed, one recent report indicated that loss of Foxp3 in Treg cells and acquisition of IFN- γ may result in the conversion of suppressor T cells into highly autoaggressive lymphocytes (exFoxp3⁺ cells), which can favor the development of autoimmune conditions (Tsuji et al., 2009; Zhou et al., 2009). Importantly, Toulza et al. (2008) 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, 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. Additionally, functional impairment of CD4⁺Foxp3⁺ Treg cells was observed in mice that were transgenic mice for the *HTLV-1 bZIP factor (HBZ)* gene, which encodes the minus strand of HTLV-1 (Satou et al., 2011). These findings support the hypothesis that HTLV-1 may be one of the exogenous retrovirus genes responsible for immune dysregulation through interference of CD4⁺CD25⁺ Treg cell function. This hypothesis is currently under investigation to elucidate the precise molecular mechanisms by which HTLV-1 influences the fate and function of CD4⁺CD25⁺CCR4⁺ T cells, especially Foxp3⁺ Treg cells.

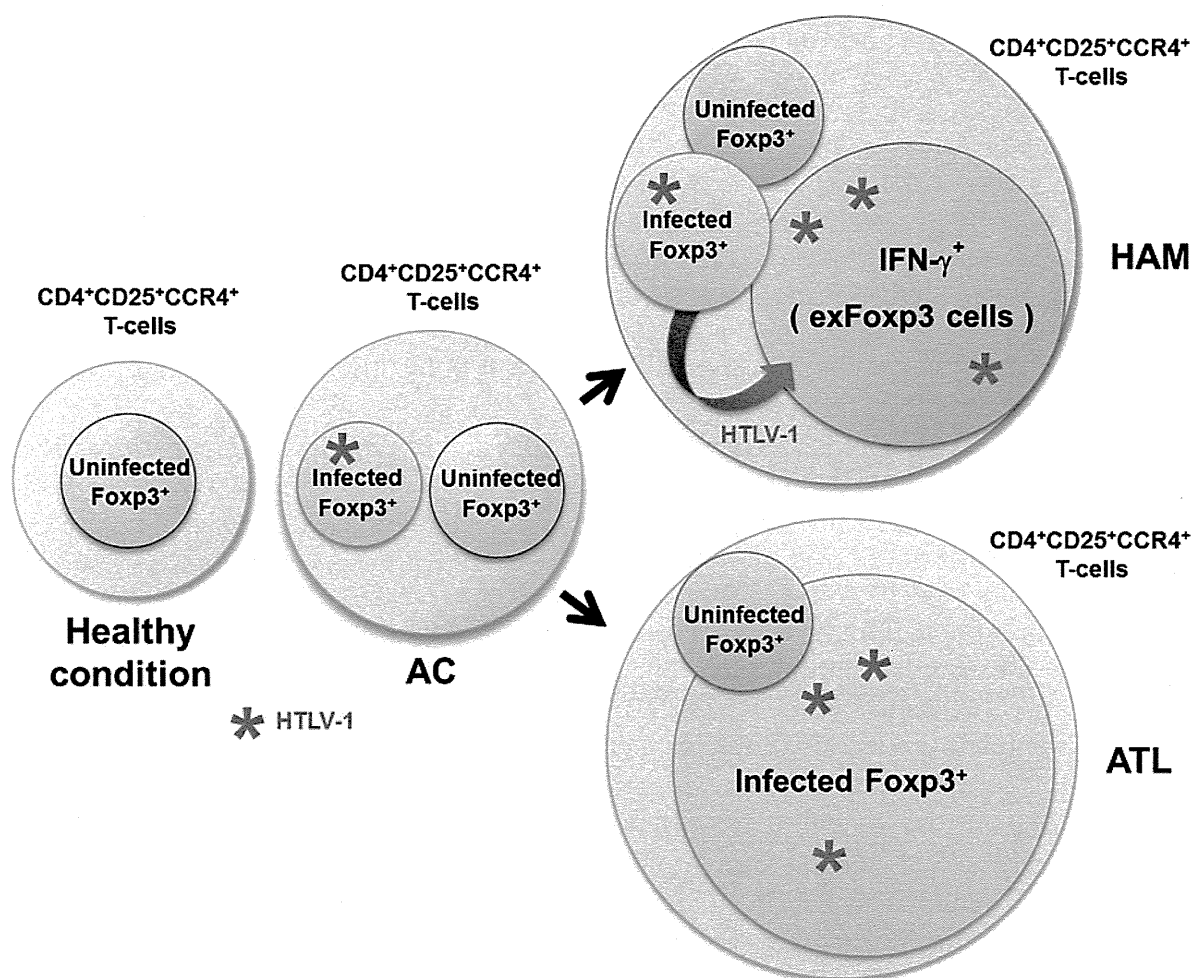


Fig. 1. Cellular components of CD4⁺CD25⁺CCR4⁺ T cells in healthy individuals, asymptomatic carriers, ATL, and HAM/TSP patients.

3. Abnormality of cytotoxic T lymphocyte (CTL) response

CD8⁺ Cytotoxic T lymphocyte (CTL) responses are an effective host defense system against all virus infections and malignancies. CTLs act by killing autologous cells that express viral

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)-1 α , MIP-1 β , 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-1 α and 1 β 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 $\gamma\delta$ 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 $\gamma\delta$ 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 $\gamma\delta$ 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 V α 24J α 18 TCR chain that preferentially associates with V β 11; 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.

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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-1-associated 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., Uchimaruru, 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 CD127^{low} expressing CD4⁺CD25^{high} T regulatory cells is inversely correlated with human T lymphotropic 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., Kuylensstierna, C., Gonzalez, V.D., Andersson, S.K., Bosnjak, L., Sönnernborg, 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. & Dokh elar, 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-I-associated 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, 1215-21
- Yamano, 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, virus-specific 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

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Glossary

Cytotoxic T cell A cytotoxic T cell belongs to a subgroup of T lymphocytes with CD8 receptor that are antigen-specific and capable of inducing the death of virus-infected somatic or tumor cells.

Gliosis Gliosis is the process of scarring in the central nervous system, caused by a proliferation of astrocytes.

Oligoclonal band Oligoclonal bands are bands of immunoglobulins that are seen when a blood serum (or plasma) or cerebrospinal fluid (CSF) is analyzed by protein electrophoresis. The presence of oligoclonal bands

in CSF but not in blood serum (or plasma) means the production of immunoglobulins in central nervous system, that is, inflammation in the central nervous system.

Provirus A provirus is the form of the virus which is capable of being integrated into the chromosome of the host cell.

Spastic paraparesis Mild or moderate loss of motor function accompanied by spasticity in the extremities mainly caused by central nervous system (brain and spinal cord) diseases.

Human T-lymphotropic virus type-1 (HTLV-1) belongs to the *Deltaretrovirus* genus of the Orthoretrovirinae subfamily and infects 10–20 million people worldwide. HTLV-1 can be transmitted through sexual contact, intravenous drug use, and breastfeeding from mother to child. The infection is endemic in south-west Japan, the Caribbean, sub-Saharan Africa, South America, with smaller foci in Southeast Asia, South Africa, and northeastern Iran. HTLV-1 was initially isolated in 1980 from two T-cell lymphoblastoid cell lines and the blood of a patient originally thought to have a cutaneous T-cell lymphoma. It was the first human retrovirus ever associated with a human cancer. Three years before the isolation of HTLV-1, a Japanese group reported adult T-cell leukemia (ATL), a rare form of leukemia endemic to southwest Japan, as a distinct clinical entity. In 1981, the same group demonstrated that ATL was caused by a new human retrovirus originally termed 'ATLV'. Later, ATL and HTLV have been shown to be identical, and a single name HTLV-1 has been adopted. In the mid-1980s, epidemiological data linked HTLV-1 infection with a chronic progressive neurological disease, which was termed 'tropical spastic paraparesis (TSP)' in the Caribbean and 'HTLV-1 associated myelopathy (HAM)' in Japan. HTLV-1-positive TSP and HAM were subsequently found to be clinically and pathologically identical and the disease was given a single designation as HAM/TSP. HTLV-1 can cause other chronic inflammatory diseases such as uveitis, arthropathy, pulmonary lymphocytic alveolitis, polymyositis, Sjögren syndrome, and infective dermatitis. Only approximately 2–3% of infected persons develop ATL and another 0.25–4% develop chronic inflammatory diseases, while the majority of infected individuals remain lifelong asymptomatic carriers (ACs). Thus, the viral, host, and environmental risk factors, as well as the host immune response against HTLV-1 infection, appear to regulate in the development of HTLV-1-associated diseases. For over two decades, the investigation of HTLV-1-mediated pathogenesis has focused on Tax, an HTLV-1-encoded viral oncoprotein. Tax activates many cellular genes by binding to groups of transcription factors and coactivators and is necessary and

sufficient for cellular transformation. However, recent reports have identified another regulatory protein, HTLV-1 basic leucine zipper factor (HBZ), that plays a critical role in the development of ATL and HAM/TSP.

HTLV-1-Associated Diseases**Adult T-cell leukemia**

ATL is a fatal malignancy of mature CD4+ T cells. It arises in only a small proportion of HTLV-1-infected people (1–5% of infected individuals) after long latency periods following primary infection. ATL shows diverse clinical features, but can be divided into four clinical subtypes: smoldering, chronic, lymphoma, and acute. Each subtype is directly correlated with the prognosis of patients: the smoldering and chronic types are indolent, while the acute and lymphoma types are aggressive and characterized by resistance to chemotherapy and poor prognosis. Development of ATL is characterized by infiltration of various tissues with circulating ATL cells, called 'flower cells', which have conspicuous lobulated nuclei. These cells cause further symptoms including lymphadenopathy, lytic bone lesions, skin involvement, hepatosplenomegaly, and hypercalcemia. Laboratory findings of ATL patients typically reveal a marked leukocytosis, hypercalcemia, high serum levels of lactate dehydrogenase (LDH), and a soluble form of interleukin-2 receptor (IL-2R). In cohort studies of HTLV-1 carriers, the risk factors for ATL appeared to include vertical infection (mother to child transmission), male gender, older age, and increasing numbers of abnormal lymphocytes. Since ATL occurs mainly in vertically infected individuals, but not in those who become infected later in life, the impairment of HTLV-1-specific T-cell responses caused by vertical HTLV-1 infection has been suggested as a possible cause of disease development. The HTLV-1-specific cytotoxic T-cell (CTL) responses from ATL patients are significantly lower than that of HAM/TSP patients. However, insufficient HTLV-1-specific T-cell responses might also occur during and after the onset of

ATL. Although ATL has a poor prognosis, recent advances in its treatment have led to significant gains in response rates and survival. Accumulating evidence suggests that allogeneic bone marrow transplantation and allogeneic peripheral blood stem cell transplantation are potent therapies for aggressive ATL (i.e., the acute and lymphoma type). The combination of the antiretroviral agent zidovudine (AZT) and interferon- α (IFN- α) is also beneficial for overall survival in smoldering and chronic (i.e., indolent) ATL, although its efficacy has not yet been confirmed in well-designed prospective studies.

p0040 Since the discovery of HTLV-1, the viral transactivator Tax has been viewed as critical for leukemogenesis, due to its pleiotropic effects on both viral and many cellular genes responsible for cell proliferation, genetic instability, dysregulation of the cell cycle, and apoptosis. However, Tax expression is not detected in about 60% of freshly isolated samples from ATL cases. Recently, the expression of another regulatory protein, HBZ, has been reported in association with all ATL cases. This protein, which is encoded in the minus or antisense strand of the virus genome, promotes proliferation of ATL cells and induces T-cell lymphomas in CD4⁺ T cells by transgenic expression, indicating involvement of HBZ expression in the development of ATL. In addition, among the HTLV-1-encoded viral genes, only the HBZ gene sequence remains intact, unaffected by nonsense mutations and deletion. Thus, HBZ expression is indispensable for proliferation and survival of ATL cells and HTLV-1-infected cells, and Tax expression is not always necessary for the development of ATL.

s0015 HTLV-1-Associated Myelopathy/Tropical Spastic Paraparesis

p0045 HAM/TSP is a chronic progressive myelopathy characterized by spastic paraparesis, sphincter dysfunction, and mild sensory disturbance in the lower extremities. In addition to neurological symptoms, some HAM/TSP cases also exhibit autoimmune-like disorders, such as uveitis, arthritis, T-lymphocyte alveolitis, polymyositis, and Sjögren syndrome. To date, more than 3000 cases of HAM/TSP have been reported in HTLV-1-endemic areas. Sporadic cases have also been described in nonendemic areas such as the United States and Europe, mainly in immigrants from an HTLV-1-endemic area. The lifetime risk of developing HAM/TSP is different among ethnic groups, ranging between 0.25% and 4%. The annual incidence of HAM/TSP is higher among Jamaican subjects than among Japanese subjects (20 vs. three cases/100 000 population), with a 2 to 3 times higher risk for women in both populations. The period from initial HTLV-1 infection to the onset of HAM/TSP is assumed to range from months to decades, a shorter time than for ATL onset. HAM/TSP occurs both in vertically infected individuals and in those who become infected later in life (i.e., through sexual contact (almost exclusively from male to female), intravenous drug use, contaminated blood transfusions, etc.). The mean age at onset is 43.8 years and, like other autoimmune diseases, the frequency of HAM/TSP is higher in women than in men (the male to female ratio of occurrence is 1:2.3).

p0050 The essential histopathological feature of HAM/TSP is a chronic progressive inflammation in the spinal cord, predominantly at the thoracic level. The loss of myelin sheaths and axons in the lateral, anterior, and posterior columns is

associated with perivascular and parenchymal lymphocytic infiltration, reactive astrocytosis, and fibrillary gliosis. In addition to HTLV-1 antibody positivity, other laboratory findings of HAM/TSP include the presence of atypical lymphocytes (the so-called flower cells) in peripheral blood and cerebrospinal fluid (CSF), a moderate pleocytosis, and raised protein content in CSF. Oligoclonal bands, raised concentrations of inflammatory markers such as neopterin, tumor necrosis factor (TNF)- α , IL-6 and IFN- γ , and an increased intrathecal antibody synthesis specific for HTLV-1 antigens have also been described in CSF of HAM/TSP patients.

A previous population association study in HTLV-1 endemic in southwest Japan revealed that one of the major risk factors is the HTLV-1 proviral load (PVL), as the PVL is significantly higher in HAM/TSP patients than in ACs. A high PVL was also associated with an increased risk of progression to disease. Higher PVL in HAM/TSP patients than in ACs was also observed in other endemic areas such as the Caribbean, South America, and the Middle East. In southwest Japan, an association was suggested between possession of the HLA-class I genes HLA-A*02 and Cw*08 and a statistically significant reduction in both PVL and the risk of HAM/TSP. By contrast, possession of HLA-class I HLA-B*5401 and class II HLA-DRB1*0101 predisposed patients in the same population to HAM/TSP. Since the function of class I HLA proteins is to present antigenic peptides to CTL, these results imply that individuals with HLA-A*02 or HLA-Cw*08 mount a particularly efficient CTL response against HTLV-1, which may be an important determinant of HTLV-1 PVL and the risk of HAM/TSP.

To date, no generally agreed standard treatment regimen has been established for HAM/TSP, as no treatment for HAM/TSP has proven to be consistently effective and long term. Therefore, current clinical practice for treatment of HAM/TSP is based on case series and open, nonrandomized uncontrolled studies. Although mild to moderate beneficial effects have been reported with corticosteroids, immunosuppressants, high-dose intravenous gammaglobulin, antibiotics (erythromycin and fosfomycin), and vitamin C, the clinical benefits are only transient and limited. The complications of steroid use limit their use particularly in postmenopausal females, who are at higher risk of developing HAM/TSP. Only three randomized placebo-controlled trials have been conducted for HAM/TSP treatment. These studies indicate that IFN- α is an effective therapy, with an acceptable side-effects profile. By contrast, no evidence yet exists of any benefit of zidovudine plus lamivudine for treating HAM/TSP. More clinical trials with adequate power are needed in the future.

Other HTLV-1-Associated Diseases

HTLV-1 has been implicated in the pathogenesis of other inflammatory disorders such as uveitis, arthropathy, infective dermatitis, pulmonary lymphocytic alveolitis, polymyositis, Sjögren syndrome, and autoimmune thyroid diseases, based on the higher HTLV-1 PVL and the higher seroprevalence in patients than in ACs. However, direct evidence for an association between these disorders and HTLV-1 infection is still lacking. Nonetheless, HTLV-1 may be a significant trigger for the development of these autoimmune disorders.