
T-CELL LEUKEMIA

Edited by **Olga Babusikova, Sinisa Dovat**
and **Kimberly J. Payne**

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Preface

1. Introduction

T cell malignancies include a spectrum of diseases. The most common of them are: 1) Adult T-cell leukemia (ATLL); 2) Childhood T-cell Acute lymphoblastic leukemia (T-ALL); 3) Cutaneous T-cell lymphoma – that includes mycosis fungoides and Sezary syndrome; and 4) Anaplastic large cell lymphoma – anaplastic lymphoma kinase (ALK)-positive. Although these diseases are distinct clinical entities, they all pose challenging treatment problems for oncologists. Many of these illnesses carry a poor prognosis, and novel treatment modalities are essential to improve survival. Over the last 10 years, significant progress has been made in understanding the molecular pathogenesis of these diseases, which promises to lead to novel targeted chemotherapeutic agents. The purpose of this book is to provide a comprehensive review of the scientific advances in T-cell malignancies and to highlight the most relevant findings that will help the reader understand both basic mechanisms of the disease and future directions that are likely to lead to novel therapies. In order to assure a thorough approach to these problems, contributors include basic scientists, translational researchers and clinicians who are experts in this field. Thus, the target audience for this book includes both basic scientists who will use this book as a review of the advances in our fundamental knowledge of the molecular mechanisms of T-cell malignancies, as well as clinicians who will use this book as a tool to understand rationales for the development of novel treatments for these diseases.

2. Adult Human T-Cell Leukemia and Molecular Mechanisms of HTLV-1

The first chapter, “Adult Human T cell Leukemia” by Herbeuval J-P., provides an overview of human ATLL. This chapter spans the role of HTLV-1 virus, including its genomic and epidemiological characteristics; the clinical picture and treatment of ATLL, and immune response to HTLV-1 with a brief overview of the role of specific types of immune cells. This chapter gives a comprehensive review of ATLL, with sufficient depth of knowledge for practical oncologists, without extensive molecular mechanistic details of the pathophysiology of the disease.

The subsequent three chapters are primarily concerned with the molecular mechanisms by which HTLV-1 causes ATLL. Chapter 2, “Human T-Cell

Lymphotropic Virus (HTLV-1) and Adult T-Cell Leukemia,” by Abbaszadegan M.R. and Gholamin M., provides an overview of the structure, epidemiology, function and pathophysiological effects of HTLV-1 in A-TALL. This chapter provides up-to-date knowledge of the first discovered human retrovirus along with its primary molecular functions. In Chapter 3, “Roles of HTLV-1 Tax in Leukemogenesis of Human T-Cells,” Nakamura M. focuses on detailed molecular mechanisms of the role of the Tax1 gene of HTLV-1 in malignant transformation and the development of ATLL. The author specifically addresses the role of Tax1 in promoting cell growth, deregulating cellular signaling, and in the modification of apoptosis and cellular immortalization. Chapter 4, “Host Immune System Abnormalities Among Patients with Human T-Lymphotropic Virus Type 1 (HTLV-1)-Associated Disorders” by Yagishita N., Araya N., and Sato T., centers on the immunological abnormalities of HTLV-1-positive patients. Specifically, functional changes in CD4⁺ T cells and abnormalities of the cytotoxic T lymphocyte (CTL) response, as well as abnormalities of innate immunity following HTLV-1 infection are described.

3. Signaling Pathways in T-Cell Malignancies

The next four chapters describe the role of specific genes or pathways in the pathogenesis of ATLL, T-ALL, and other T-cell malignancies. Chapter 5, “Constitutive Activation of the JAK/STAT and Toll-Like Receptor Signaling Pathways in Adult T-Cell Leukemia/Lymphoma” by Tsukada J., et al., focuses on the functional specificity of these pathways in HTLV-1 infected cells and their role in malignant transformation. In Chapter 6, “The Role of Ikaros in T-ALL,” Dovat S. and Payne K.J. dissect the role of the Ikaros (Ikzf1) tumor suppressor gene and its regulatory signal transduction pathways in the control of proliferation in malignant cells. The novel findings that Casein Kinase 2 (CK2) and Protein Phosphatase 1 (PP1) control the ability of Ikaros to regulate tumor suppression in T-cell leukemia are summarized. Chapter 7, “p16^{INK4A} – Connecting Cell Cycle Control to Cell Death Regulation in Human Leukemia” by Ausserlechner M.J. et al., focuses on the role of the INK4 inhibitor protein p16^{INK4A} in the regulation of cell cycle and cell survival in T-ALL. The role of the INK4A gene in the generation of induced pluripotent stem cells and in hematopoietic stem cells is discussed. Chapter 8, “Accumulation of Specific Epigenetic Abnormalities During Development and Progression of T cell Leukemia/Lymphoma” by Yoshino T. et al., describes recent findings on the role of epigenetic changes in the development of T cell leukemia and in cellular proliferation. This chapter summarizes the scientific advances in epigenetic regulation of gene expression related to T cell leukemia/lymphoma, and provides an overview of the role of DNA methylation and histone modifications in the regulation of gene expression. Chapter 9, “Roles of MicroRNA In T-Cell Leukemia” by Tomita M., centers on another emerging field of molecular biology – microRNA and its role in the regulation of gene activity. This chapter gives a nice overview of the mechanism of microRNA action that is easily understandable for both basic scientists and clinicians and describes the role of microRNA in different types of T-cell malignancies.

4. Complications of T-Cell Malignancies and Experimental Therapy

The last three chapters cover the mechanisms of a common complication of ATLL – hypercalcemia, as well as a novel therapeutic approach for this disease. Chapter 10, “Mechanisms of Humoral Hypercalcemia of Malignancy in Leukemia/Lymphoma” by Rosol T.J., et al. provides an overview of hypercalcemia of malignancy. The chapter emphasizes the roles of various factors in the pathogenesis of humoral hypercalcemia of malignancies (HHM), the clinical significance of HHM in various T-cell malignancies, and therapeutic approaches to HHM. Chapter 11, “The Role of T-Cell Leukemia Translocation-Associated Gene (TCTA) Protein in Human Osteoclastogenesis” by Nanke Y. et al. concentrates on the mechanism by which the TCTA protein regulates osteoclastogenesis. The role of the receptor activator NF- κ B ligand (RANKL) in osteoclastogenesis, and recent discoveries about the role of TCTA as an inhibitor of RANKL-induced osteoclastogenesis is described in detail. Chapter 12, “Retrovirus Infection and Retinoid,” Maeda Y et al. describe retinoids as a potential novel treatment agent for ATLL. The growth inhibition effect of All-Trans Retinoic Acid (ATRA), its downregulation of IL-2R α /CD25, and its inhibition of NF- κ B transcription activity by ATRA are described. The potential clinical use of retinoids for T-cell malignancies is discussed.

5. Summary

This book provides an up-to-date overview of advances in our understanding of the pathogenesis of T cell malignancies and will serve as a tool for both basic scientists and clinicians by providing a basis for further studies and an aid in the search for more effective therapies for these challenging diseases.

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Human T-Cell Lymphotropic Virus (HTLV-1) and Adult T-Cell Leukemia

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1. Introduction

Human T-cell Lymphotropic Viruses (HTLVs) and Simian T-cell Lymphotropic Viruses (STLVs) are anciently related primate T-cell leukemia viruses (PTLVs) that share molecular and virological features. Human T-cell Lymphotropic Virus (HTLV-1) is believed to be repeatedly transmitted in separate independent events from simians to humans beginning $50,000 \pm 10,000$ years ago; this course has resulted in the formation of several viral subtypes around the world. There are four known strains of HTLV, of which HTLV-1 and HTLV-2 are the most prevalent worldwide. Newer HTLVs, HTLV-3 and 4 have been identified recently from bush meat hunters in central Africa (Matsuoka and Jeang 2007).

HTLV-1, the first human retrovirus was discovered by two independent investigating groups in 1980 and 1981. A geographical clustering of leukaemias in southwestern Japan led to the description of a unique clinical entity termed adult T-cell leukemia (ATL), where Japanese investigators identified HTLV-1 as an etiologic agent of newly described ATL, and the U.S. investigators detected HTLV-1 retrovirus in human cell lines (Yoshida 2010).

HTLV-1 belongs to the Deltaretrovirus genera of the Orthoretrovirinae subfamily, the first discovered human retrovirus, isolated in the early 1980s from peripheral blood samples of a patient with cutaneous T-cell lymphoma (Poiesz et al, 1980). It is the etiologic agent of two predominant distinct human diseases, ATL or adult T-cell leukemia lymphoma (ATLL) and a chronic, progressive demyelinating disorder known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Zanjani et al. 2010).

The major findings that support the etiologic association of HTLV-1 are: 1) All patients with ATL have antibodies against HTLV-1, 2) The areas of high incidence of ATL patients correspond closely with those of high incidence of HTLV-1 carriers, 3) HTLV-1 immortalizes human T cells *in vitro*, 4) Monoclonal integration of HTLV-1 proviral DNA was demonstrated in ATL cells. Thus, HTLV-1 is the first retrovirus directly associated with human malignancy (Takatsuki 2005). HTLV-1 is a complex leukemogenic retrovirus with a single stranded positive sense RNA genome that expresses unique proteins with oncogenic potential. HTLV-1 can infect T cells, B cells, monocytes, dendritic cells and endothelial cells with equal efficiency; yet, it can transform only primary T cells (Hanon et al. 2000).

HTLV-2 was identified in a CD8⁺ T cell line derived from a patient with a variant form of hairy T cell leukemia. Since then, HTLV-2 has not been associated with leukemia/lymphoma; nevertheless, it has been associated with a few sporadic cases of

neurological disorders and chronic encephalomyelopathy (Hjelle et al. 1992). The clinical symptoms presented are similar to those of HAM/TSP. The prevalence of HTLV-2-associated myelopathy was reported to be 1% compared to 3.7% for HTLV-1 associated HAM/TSP in the United State. Although other neurological disorders have been reported, their clear association with HTLV-2 is hampered by confounding factors such as intravenous drug use or concomitant HIV infection. To date, HTLV-3 and HTLV-4 have not been associated with any known clinical conditions (Kannian 2010).

In 1985, Gessain et al., demonstrated that 68% of patients with tropical spastic paraparesis (TSP) in Martinique had positive serology for HTLV-1. In 1986, a similar neurological condition was described in Japan and named HTLV-1 associated myelopathy (HAM). Later, Román and Osame (1988) concluded that they were dealing with the same disease, and the term HTLV associated myelopathy/tropical spastic paraparesis (HAM/TSP) came to be used. Since then, countless other diseases have been correlated with this infection: uveitis, Sjögren's syndrome, infectious dermatitis, polymyositis, arthropathies, thyroiditis, polyneuropathies, lymphocytic alveolitis, cutaneous T-cell lymphoma, strongyloidiasis, scabies, Hansen's disease and tuberculosis. The importance of the possible clinical manifestations of the HTLV virus has now become clear in several different medical specialties such as oncology, neurology, internal medicine, dermatology, and ophthalmology (Romanelli, Caramelli and Proietti 2010).

Only HTLV-1-infected individuals develop ATL, and all ATL cells contain integrated HTLV-1 provirus, supporting the causal etiology of the virus for leukaemogenesis. Nevertheless, only a small minority of HTLV-1-infected individuals progress to ATL. Indeed, the cumulative risks of developing ATL among virus carriers are estimated to be approximately 6.6% for males and 2.1% for females (Matsuoka and Jeang 2007).

A long period of latency from HTLV-1 infection to ATL development suggests a multistep process of T-lymphocyte transformation. In ATL patients, the malignant cells typically consist of oligoclonal or monoclonal outgrowths of CD4+ and CD25+ T lymphocytes carrying a complete or defective provirus of HTLV-1. Four clinical subtypes of ATL include acute, lymphoma, chronic and smoldering (Noula Shembade 2010).

2. Worldwide distribution

Approximately 15-25 million people worldwide are infected with HTLV-1. The virus is endemic in southwestern Japan, Africa, the Caribbean Islands and South America and is frequently found in Melanesia, Papua New Guinea, Solomon Islands and Australian aborigines. HTLV-1 is also prevalent in certain populations in the Middle East (Iran) and India. HTLV-2 is more prevalent among intravenous drug users (IDUs), and is endemic among IDUs in the USA, Europe, South America and Southeast Asia. HTLV-3 and HTLV-4 have been identified only in African primate hunters (Kannian 2010). HTLV-1 infection is endemic in northeastern Iran (Khorasan province) and the prevalence of HTLV-1 infection is estimated to be 2-3% in the whole population and 0.78% in blood donors (Abbaszadegan et al. 2003; Safai et al. 1996).

High prevalence rates in the general population are observed in the South of Japan (10%), in Jamaica and Trinidad and Tobago (6%). In South America (Argentina, Brazil, Colombia and Peru) a 2% prevalence of seropositivity was observed among blood donors. It is known that the prevalence of HTLV-1 in population of blood donors represents an underestimation of prevalence in the general population. In absolute terms, Brazil may have the largest number

of seropositive people in the world. In non-endemic areas, certain groups should be considered as at risk, such as immigrants from endemic areas, the sexual partners and descendants of people known to be infected, sex professionals and drug users (Romanelli, Caramelli and Proietti 2010).

HTLV-1 carriers are mostly asymptomatic in their life spans. The lifetime risks of developing ATL and HAM/TSP are about 2.5 to 5% and 0.3 to 2%, respectively (Silva et al. 2007). Among HTLV-1 infected individuals in Japan, a small proportion of carriers (6% for males and 2% for females) develop ATL. The majority of HTLV-1 carriers do not develop HTLV-1-associated diseases. The latency period from the initial infection until onset of ATL is about 60 years in Japan and 40 years in Jamaica. These determinations indicate a multistep leukemogenic mechanism in the generation of ATL.

3. Genomic structure of HTLV-1

HTLV-1 virions are complex type C particles, spherical, enveloped and 100–110 nm in diameter. The inner membrane of the virion envelope is lined by the viral matrix protein (MA). This structure encloses the viral capsid (CA), which carries two identical strands of the genomic RNA as well as functional protease (Pro), integrase (IN), and reverse transcriptase (RT) enzymes. A newly synthesized viral particle attaches to the target cell receptor through the viral envelope (Env) and enters via fusion, which is followed by the uncoating of the capsid and the release of its contents into the cell cytoplasm. The viral genome consists of a linear, positive sense, ssRNA held together by hydrogen bonds. Each monomer has about 9032 nucleotides. The 3' terminal viral genome is polyadenylated and its 5'- terminal is capped. Each unit is associated with a specific molecule of tRNA that is base paired to a region, primer binding site, near the 5' end of the RNA. Proviral forms are flanked at both termini by long terminal repeats (LTRs) of 754 nucleotides. The genomic structure encodes structural and enzymatic proteins: gag, pol, env, reverse transcriptase, protease, and integrase. In addition, HTLV-1 has a region at the 3' end of the virus, called pX, which encodes four partially overlapping reading frames (ORFs). These ORFs code for regulatory proteins which impact the expression and replication of the virus (Figure 1) (Boxus and Willems 2009).

The viral RNA is reverse transcribed into double stranded DNA by the RT. This double stranded DNA is then transported to the nucleus and becomes integrated into the host chromosome forming the provirus. The provirus contains the promoter and enhancer elements for transcription initiation in the long terminal repeats (LTR); the polyadenylation signal for plus strand transcription are located in the 3'LTR (Kannian 2010).

The initial round of HTLV-1 transcription is dependent on cellular factors. The complex retroviral genome codes for the structural proteins Gag (capsid, nucleocapsid, and matrix), Pro, polymerase (Pol) and Env from unspliced/singly spliced mRNAs. Alternatively spliced mRNA transcripts encode regulatory and accessory proteins. The two regulatory genes rex and tax are encoded by open reading frames (ORF) III and IV, respectively, and share a common doubly spliced transcript. Tax is the transactivator gene, which increases the rate of viral LTR-mediated transcription and modulates the transcription of numerous cellular genes involved in cell proliferation and differentiation, cell cycle control and DNA repair. Tax has displayed oncogenic potential in several experimental systems and is essential for HTLV-1 and HTLV-2-mediated transformation of primary human T cells. Rex acts post-transcriptionally by preferentially binding, stabilizing exporting intron-containing viral

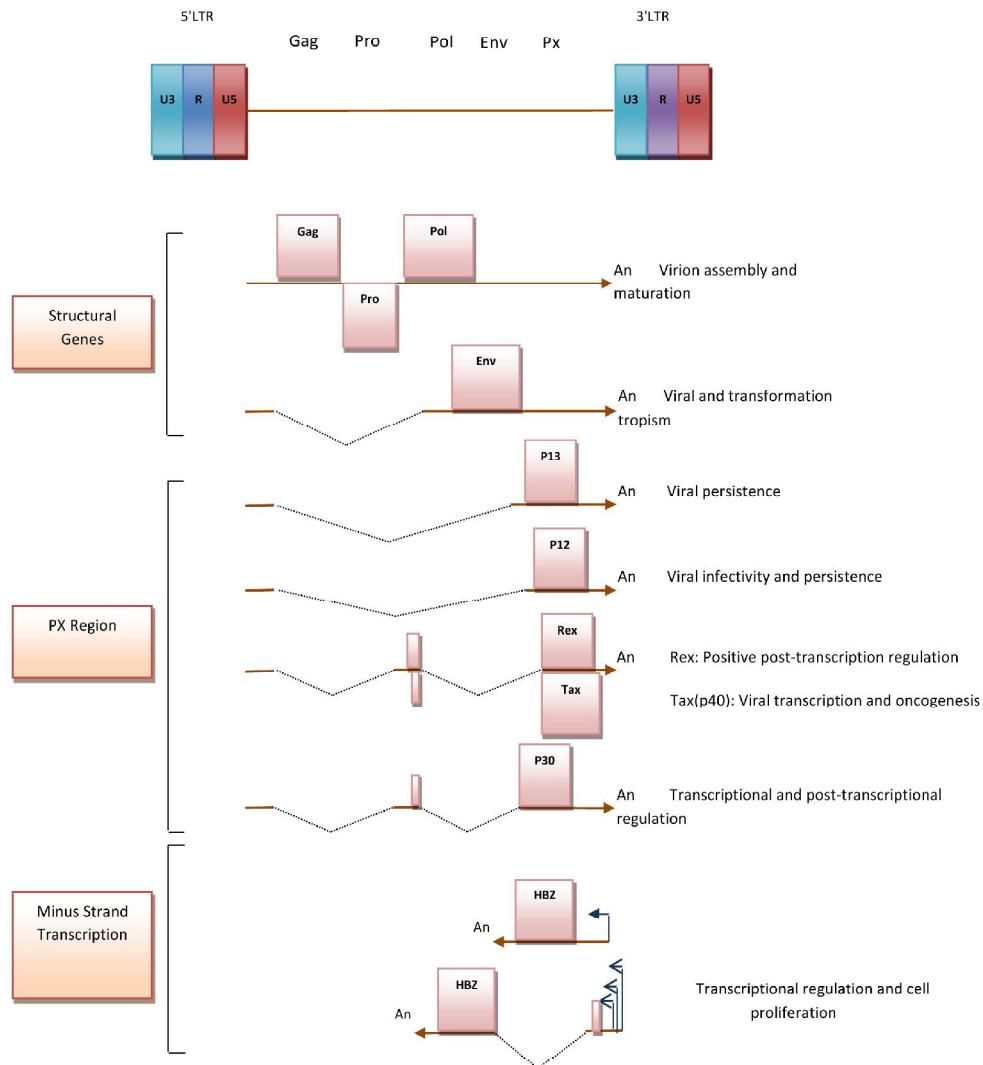


Fig. 1. HTLV-1 genome structure and gene product

mRNAs from the nucleus to the cytoplasm. The accessory genes, p12/p8 encoded by ORF I and p30/p13 encoded by ORF II is not necessary in standard immortalization assays in culture. However, these genes are essential for initiation of viral infection and the establishment of persistence in animal models. P8 is a proteolytic cleavage product of the p12 parent molecule, whereas the p13 polypeptide, comprised of the carboxy terminus of p30, is expressed from a distinct mRNA. These accessory proteins may also play a role in gene regulation and contribute to the productive infection of quiescent T lymphocytes *in vitro*. The minus strand of the proviral genome encodes several isoforms (generated from

unspliced and spliced mRNAs) of the HTLV-1 basic leucine zipper factor (HBZ). HBZ interacts with cellular factors JunB, c-Jun, JunD, cAMP response element binding (CREB) and CREB binding protein (CBP)/p300 to modulate both viral and cellular gene transcription. HBZ also plays a crucial role in T cell proliferation. Although research data indicate that Tax, among all the viral proteins, is the viral oncoprotein, but emerging data suggests a supporting role for HBZ in the oncogenic process (Kannian 2010).

4. Transmission

HTLV-1 can infect various cell types, including T-lymphocytes, B-lymphocytes, monocytes, dendritic cells (DC) and fibroblasts. Glucose transporter 1 (GLUT-1) is ubiquitously expressed cell surface receptor targeted by HTLV-1 (Manel et al. 2003). Other cell surface receptors such as neuropilin 1 (NRP1) and surface heparan sulfate proteoglycans (HSPGs) have been reported to be target of HTLV-1 and are required for efficient entry (Boxus and Willems 2009). There is also evidence for cell-type specific receptors since a recent study has reported that HTLV-1 enters DCs by binding to the receptor DC-SIGN (Noula Shembade 2010). However, the HTLV-1 provirus is mainly detected in CD4-positive lymphocytes, with about 10% in CD8-positive T-lymphocytes. This situation possibly arises because of Tax transformation of CD4-positive T-lymphocytes *in vivo* causing enhanced proliferation and suppressed apoptosis. In HTLV-1-infected individuals, no virions are detected in the serum. In addition, the infectivity of free virions is very poor compared with that of infected cells suggesting that HTLV-1 spreads through cell-to-cell transmission, rather than by free virions. *In vitro* analyses of HTLV-1 infected cells revealed that HTLV-1-infected cells form "virological synapses" with uninfected cells. The viral proteins Gag and Env, viral RNA and microtubules are accumulated as an infected cell is in contact with a target cell, and the viral complex subsequently transfers into the target cell. HTLV-1 also spreads in a cell- to-cell manner via such virological synapses *in vivo* (Igakura et al. 2003).

In either route, HTLV-1-infected cells are essential for transmission. This was supported by the findings that fresh frozen plasma from carriers did not cause transmission and freeze-thawing of breast milk reduced vertical transmission (Matsuoka 2005).

Transplacental transmission is also suspected. Cellular blood products are the main source of transfusion- associated HTLV transmission, whereas fresh frozen plasma, cryoprecipitate, or coagulation factor concentrates appear not to cause infection (Abbaszadegan et al. 2003).

The efficiency of virus transmission is from males to females during sexually active years causing a higher seroprevalence of about more than twice in females. The HTLV-1 infection tends to be more within family members and three to four times greater than its rate in general population. It is proposed that repeated close contact and shared environment could be significant in HTLV-1 transmission (Rafatpanah et al. 2006). Viral antigens expressed by infected cell are quickly targeted by cytotoxic T cells; hence the viral load is maintained predominantly by cells harboring silent provirus spread by mitotic transmission. HTLV-1 transmission by free virions is very inefficient, at least in T cells, however, recent studies indicate that cell-free HTLV-1 virions are highly infectious for DCs (Noula Shembade 2010).

4.1 Transmission of HTLV-1 occurs through three main routes

HTLV-1 is mainly transmitted via three routes: 1) mother- to-infant transmission, mainly through breast feeding; 2) sexual transmission, mainly from male-to- female; and 3) parenteral transmission (blood transfusion or intravenous drug use).

Mother to child: One of the main modes of HTLV-1 transmission from mother to child is by breast feeding. Studies in Japan showed that the prevalence of HTLV-1 infection in children of carrier mothers was significantly higher (21%) than in children in the general population (1%). More than 85% of infected mothers had infected their children. The length of breast-feeding affects the risk of HTLV-1 transmission. The duration of breast-feeding affects the risk of HTLV-1 transmission. HTLV-1 antigen in cord blood lymphocytes of babies born to healthy carriers raised a possibility to consider intrauterine transmission as an alternative pathway. However, the HTLV-1 provirus in the cord blood circulation is derived from migrated maternal cells that are not a part of blood circulation of the baby. Thus, intrauterine transmission could not be a major pathway of transmission.

Only ~3%–4% of children become infected if they are not breast-fed or are breast-fed for <6 months, and the transmission risk increases with the duration of breast-feeding. The cumulative risk of infection in children who are regularly breast-fed is ~20%. Furthermore, the transmission risk increases with the amount of provirus in breast milk. Breast milk proviral levels also correlate with proviral levels in maternal peripheral blood mononuclear cells (PBMCs) and with antibody titers. It is therefore not surprising that the transmission risk correlates with proviral and antibody levels in maternal peripheral blood. However, this is contradictory to the fact that the majority of children who are breastfed for long periods do not become infected. HIV can also be transmitted by breast-feeding, however, it is more commonly transmitted in utero or perinatally. As with HTLV-1, transmission occurs in proportion to the duration of breast-feeding, and the risk increases when mothers have high HIV provirus levels in breast milk, which are also directly correlated with peripheral blood provirus levels. Mother- to-child in utero and perinatal HIV transmission was more likely when children were more concordant with their mothers in HLA class I type. Children with HLA class I type A*02 (later reported to be the A2 supertype) have a decreased risk of early infection. HLA class I type variability is not associated with HIV transmission via breast-feeding, whereas, with HTLV-1, vertical transmission almost always occurs via breast-feeding. However, unlike HIV, HTLV-1 is transmitted primarily by cell- to-cell contact rather than by free virus. We reasoned that factors affecting cellular immunity might be more important for the vertical transmission of HTLV-1 than for HIV (Biggar et al. 2006).

Transmission rates are 16% for children born to infected mothers, 27% for children nursed by infected mothers for more than three months and 5% for children nursed by infected mothers for less than three months (Ureta-Vidal et al. 1999). It is interesting that HTLV is transmitted to about 13% of bottle-fed children from their infected mother suggesting a different route than breast-feeding. The infants seroconvert within 1-3 years of age (Ureta-Vidal et al. 1999). The infants seroconvert within 1-3 years of age (Ureta-Vidal et al. 1999).

Sexual: HTLV-1 can also be transmitted through sexual contact. Heterosexual transmission is able to introduce HTLV-1 infection into previously uninfected groups. Transmission from man to woman is more frequent (60%) than woman to man (0.4%). Like HIV, HTLV-1 can be transmitted through homosexual activity. Predisposing factors associated with sexual transmission include the presence of genital ulcers, high viral loads and high antibody titers in the donor (Kaplan et al. 1996). Sexual transmission is a more common mode in non-drug user sexual partners of IDUs than parenteral transmission. Among IDUs, blood and blood products are the most significant source of infection (Roucoux and Murphy 2004).

Blood transfusion: is the third mode of HTLV-1 transmission. The proviral DNA in donor's blood lymphocytes acts as an infectious agent. The probability of seroconversion in a recipient

of contaminated blood is about 44%. Thus, it is essential to have an efficient blood screening system for HTLV-1 in endemic areas to limit the HTLV-1 transmission. Whole blood components, platelets and packed red blood cells, but not fresh frozen plasma, are the sources of virus transmission. White blood cells are reservoirs of HTLV-1. The probability of transmission of HTLV-1 decreases, if infected units of blood are stored for more than one week HTLV-1. Approximately 12% of HTLV infections occur by blood transfusion. Unlike HIV-1, whole cell transfusion is required for transmission of the virus, with a seroconversion rate of approximately 50%. The development of HAM/TSP has been noted as early as six months after transfusion of an individual with infected blood. In 1988, concerns about transmission of HTLV through blood components led to mandatory blood donor screening for HTLV resulting in a significant decrease in transmission via this mode. Cell-free infection with HTLV-1 is very inefficient; and efficient transmission depends on cell-to-cell transfer through direct cell contact, polarization of the microtubule-organizing center (MTOC), which is triggered by Tax, and the formation of a virological synapse, which allows the entry of viral particles, viral proteins and genomic RNA into fresh target cells. Similar to HIV-1 infection, dendritic cells (DCs) have been demonstrated to play a biphasic role in cell-to-cell transmission of HTLV-1. DCs can capture and transfer the virions to fresh T cells in a trans fashion or transmit de novo synthesized virions upon infection to fresh T cells in a cis fashion (Kannian 2010). Transmission via organ transplantation has been described and is associated with rapidly progressing HAM/TSP, possibly because of the immunosuppression that transplant patients undergo (Romanelli, Caramelli and Proietti 2010).

Contaminated needles from Drug addicts: HTLV-1 can also be transmitted by sharing of needles among drug addicts (Rafatpanah et al. 2006). In the United States, HTLV infection of intravenous drug users (IVDUs) was first reported from Queens, New York, and was mainly attributed to HTLV-II (Robert-Guroff et al. 1986). Subsequently, others have reported prevalence rates of HTLV antibody among IVDUs ranging from 0.4% to 24.0% (Lee et al. 1990).

5. Diagnosis

HTLV-1 is usually detected by carrying out laboratory tests because of clinical suspicion, screening at the blood bank or due to concerns by family members of HTLV-1 positive patients. The antibodies can be detected by enzyme-linked immunosorbent assay (ELISA). ELISA kits have high sensitivity and low specificity; thus, it may not be a reliable screening tool. Therefore, positive ELISA results should be confirmed by western blot analysis and or polymerase chain reaction (PCR) (Andrade et al. 2010).

For the diagnosis of HTLV-1/2 infection, the first immunoassays used HTLV-1 whole-viral lysate as the only antigen. Then, assays were based on recombinant and/or synthetic peptide antigens only or in combination with viral lysates. Furthermore, HTLV-2 specific antigens were included, which improved the sensitivity for detection of HTLV-2 antibodies. At present, the initial diagnosis of HTLV-1/2 infection is based mainly on screening for antibodies by ELISA. Even the lack of Food and Drug Administration (FDA) licensure for HTLV-1/2 Western blot (WB) assay, it is generally applied to all repeatedly reactive samples for further confirmation of HTLV-1/2 infection (CDC, 1988). The WB assay reduces the number of false positive transmembrane results thereby increasing the specificity for serological confirmation of HTLV-1/2. This assay contains viral lysates and recombinant

proteins. MTA-1 is a unique HTLV-1 envelope recombinant protein (rgp46-I), K-55 is a unique HTLV-2 envelope recombinant protein (rgp46-II), and GD21 is a common yet specific HTLV-1 and HTLV-2 epitope recombinant envelope protein. An HTLV-1 positive sample was considered when there were bands for the gag proteins p19 and p24, and the env proteins GD21 and rgp46-I; HTLV-2 positive if p24, GD21, and rgp46-II bands were present; an indeterminate sample when there were specific bands for the virus that did not meet the HTLV-1/2 positivity criteria, and a negative result for those samples that did not exhibit any specific band. In some cases, however, it is necessary to perform a complementary assay such as a nested-polymerase chain reaction (nested-PCR) in order to confirm true HTLV-1/2 infection and to obtain a conclusive diagnosis. When WB is used for confirmation, a significant proportion of the samples reports indeterminate results, ranging from 0.02% in non-endemic areas to 50% in endemic ones, although it has been observed that indeterminate samples could result in true HTLV-1/2 infection, even in non-endemic areas. Several studies have shown that most low-risk HTLV-seroindeterminate and asymptomatic individuals are negative for HTLV-1/2 infection after testing with a highly sensitive nested-PCR. It is known that the use of highly efficient screening assays may reduce significantly false reactive results, diminishing the amount of samples further submitted to WB and/or nested-PCR analysis for confirmation. One of the strategies proposed to reduce the number of samples requiring confirmatory testing is the use of a dual ELISA algorithm (Yoshida 2010).

A pitfall in ELISA-based immunoassay may exist in HTLV-1 detection due to truncated MTA-1 envelope glycoprotein. This report describes experiments designed to determine whether some discrepancies between ELISA and PCR results could be due to truncation of immunodominant epitopes using immunoassay method. Recombinant envelope glycoprotein is used in production of diagnostic enzyme-linked immunosorbent assay (ELISA) kit. There are some reports that a significant percentage of Iranian HTLV-1 infected patients showed no seroreactivity with MTA-1 peptide, while HTLV-1 had been confirmed by PCR detection methods or ELISA kits containing a cocktail of HTLV-1 specific peptides. Some discrepancies between ELISA and PCR results could be due to truncation of immunodominant epitopes using immunoassay method. This is because of an insertion of a cytosine in position 271 causing a stop codon in the MTA-1 protein translation. SDS-PAGE analysis also failed to reveal the presence of the desired protein. Subjects with a mutant HTLV-1 env gene were shown to be seronegative using ELISA, but positive with PCR (Abbaszadegan et al. 2008).

Three diagnostic criteria for ATL have been defined. The first is the presence of morphologically proven lymphoid malignancy with T-cell surface antigens (typically CD4+, CD25+). These abnormal T lymphocytes have hyperlobulated nuclei in acute ATL and are known as "flower cells." On the other hand, in the indolent types of ATL, smoldering and chronic types, the abnormality of the nuclear shape is generally milder than that in the acute form of the disease. The second criteria is the presence of antibodies to HTLV-1 in the sera, and the third is the demonstration of monoclonal integration of HTLV-1 provirus in tumor cells by Southern blotting (Yasunaga and Matsuoka 2007).

6. HTLV-1 and the host immune system

HTLV-1 is a complex retrovirus that may have been transmitted to humans from monkeys more than ten thousands years ago. The human host has several immune mechanisms that

eliminate foreign pathogens, and like other successful pathogens, HTLV-1 must have strategies for escaping the host immune response.

Like HIV, HTLV-1 mainly infects CD4 T cells, which are the central regulators of the acquired immune response. To establish persistent infection, HTLV-1 perturbs the regulation of CD4 T cells, sometimes leading to diseases such as ATL or chronic inflammatory diseases such as HAM/TSP, uveitis, arthritis, and alveolitis. Since the discovery of HTLV-1, extensive studies have been performed using various experimental approaches to elucidate the exact pathogenesis of this virus. However, the nature of HTLV-1 pathogenesis still remains elusive. This problem is a serious obstacle to establishing effective therapies for HTLV-1 associated diseases. Precise insight into HTLV-1 mediated pathogenesis requires careful consideration of the host cells and the effect HTLV-1 has on them. A better understanding of the interactions between HTLV-1 and the host immune system should provide additional clues to effective therapies for HTLV-1- associated diseases (Satou and Matsuoka 2010).

The host immune system, especially the cellular response, against HTLV-1 exerts critical control over virus replication and the proliferation of infected cells. CTLs against the virus have been extensively studied, and Tax protein was found to be the dominant antigen recognized by CTLs *in vivo*. HTLV-1-specific CD8-positive CTLs are abundant and chronically activated. The paradox is that the frequency of Tax-specific CTLs is much higher in HAM/TSP patients than in carriers. Since the provirus load is higher in HAM/TSP patients, this finding suggests that the CTLs in HAM/TSP cannot control the number of infected cells. One explanation for this is that the CTLs in HAM/TSP patients show less efficient cytolytic activity toward infected cells, whereas CTLs in carriers can suppress the proliferation of infected cells. Hence, the gene expression profiles of circulating CD4+ and CD8+ lymphocytes were compared between carriers with high and low provirus loads. The results revealed that CD8+ lymphocytes from individuals with a low HTLV-1 provirus load show higher expressions of genes associated with cytolytic activities or antigen recognition than those from carriers with a high provirus load. Thus, CD8+ T-lymphocytes in individuals with a low provirus load successfully control the number of HTLV-1-infected cells due to their higher CTL activities. Thus, the major determinant of the provirus load is thought to be the CTL response to HTLV-1. As mentioned above, the provirus load is considered to be controlled by host factors. Considering that the cellular immune responses are critically implicated in the control of HTLV-1 infection, human leukocyte antigen (HLA) should be a candidate for such a host genetic factor. From analyses of HAM/TSP patients and asymptomatic carriers, HLA-A02, and Cw08 are independently associated with a lower provirus load and a lower risk of HAM/TSP. In addition, polymorphisms of other genes including TNF- α , SDF-1, HLA-B54, HLA-DRB-10101 and IL-15 are also associated with the provirus load, however with a lower significance than with HLA-A02, and Cw08. Regarding the onset of ATL, only a polymorphism of TNF- α gene was reported to show an association. However, familial clustering of ATL cases is a well-known phenomenon, strongly suggesting that genetic factors are implicated in the onset of ATL. Spontaneous remission is more frequently observed in patients with ATL than those with other hematological malignancies. Usually, this phenomenon is associated with infectious diseases, suggesting that immune activation of the host enhances the immune response against ATL cells. If the immune response against HTLV-1 is implicated in spontaneous remission, this suggests the

possibility of immunotherapy for ATL patients by the induction of an immune response to HTLV-1, for example via antigen-stimulated dendritic cells. Immunodeficiency in ATL patients is pronounced, and results in frequent opportunistic infections by various pathogens, including *Pneumocystis carinii*, cytomegalovirus, fungus, *Strongyloides* and bacteria, due to the inevitable impairment of the T-cell functions. To a lesser extent, impaired cell-mediated immunity has also been demonstrated in HTLV-1 carriers. Such immunodeficiency in the carrier state may be associated with the leukemogenesis of ATL by allowing the proliferation of HTLV-1-infected cells. A prospective study of HTLV-1-infected individuals found that carriers who later develop ATL have a higher anti-HTLV-1 antibody and a low anti-Tax antibody level for up to 10 years preceding their diagnosis. This finding indicates that HTLV-1 carriers with a higher anti-HTLV-1 titer, which is roughly correlated with the HTLV-1 provirus load and a lower anti-Tax reactivity, may be at the greatest risk of developing ATL. The anti-HTLV-1 antibody and soluble IL-2 receptor (sIL-2R) levels are correlated with the HTLV-1 provirus load, and a high antibody titer and high sIL-2R level are risk factors for developing ATL among carriers. Taken together, these findings suggest that a higher proliferation of HTLV-1-infected cells and a low immune response against Tax may be associated with the onset of ATL. Given these findings, potentiation of CTLs against Tax via a vaccine strategy may be useful for preventing the onset of ATL. EBV-associated lymphomas frequently develop in individuals with an immunodeficient state associated with transplantation or AIDS. This has also been reported in an ATL patient. Does such an immunodeficient state influence the onset of ATL? Among 24 patients with post-transplantation lymphoproliferative disorders (PT-LPDs) after renal transplantation in Japan, 5 cases of ATL have been reported. Considering that most PT-LPDs are of B-cell origin in Western countries, this frequency of ATL in Japan is quite high. Although the high HTLV-1 seroprevalence is due to blood transfusion during hemodialysis, the immunodeficient state during renal transplantation apparently promotes the onset of ATL. In addition, when experimental allogeneic transplantation was performed to 12 rhesus monkeys and immunosuppressive agents (cyclosporine, prednisolone or lymphocyte-specific monoclonal antibodies) were administered to prevent rejection, 4 of the 7 monkeys that died during the experiment showed PT-LPDs. Importantly, the HTLV-1 provirus was detected in all PT-LPD samples. These observations emphasize that transplantation into HTLV-1-infected individuals or from HTLV-1 positive donors require special attention. Although the mechanism of immunodeficiency remains unknown, some previous reports have provided important clues. One mechanism for immunodeficiency is that HTLV-1 infects CD8-positive T-lymphocytes, which may impair their functions. Indeed, the immune response against Tax via HTLV-1-infected CD8-positive T-cells renders these cells susceptible to fratricide mediated by autologous HTLV-1-specific CD8-positive T-lymphocytes. Fratricide among virus-specific CTLs could impair the immune control of HTLV-1. Another mechanism for immunodeficiency is based on the observation that the number of naive T-cells decreases in individuals infected with HTLV-1 via decreased thymopoiesis [48]. In addition, CD4⁺ and CD25⁺ T-lymphocytes are classified as immunoregulatory T-cells that control the host immune system. Regulatory T-cells suppress the immune reaction via the expression of immunoregulatory molecules on their surfaces. The FOXP3 gene has been identified as a master gene that controls gene expressions specific to regulatory T-cells. FOXP3 gene transcription can be detected in some ATL cases (10/17;

59%). Such ATL cells are thought to suppress the immune response via expression of immunoregulatory molecules on their surfaces, and production of immunosuppressive cytokines (Matsuoka 2005).

7. Mechanism of oncogenesis by HTLV 1

In 1977, Takatsuki et al. reported ATL as a distinct clinical entity. This disease is characterized by its aggressive clinical course, infiltrations into skin, liver, gastrointestinal tract and lung, hypercalcemia and the presence of leukemic cells with multilobulated nuclei, flower cell (Figure 2). The linkage between ATL and HTLV-1 was proven by Hinuma et al., who demonstrated the presence of an antibody against HTLV-1 in patient sera. Thereafter, Seiki et al. determined the whole sequence of HTLV-1 and revealed the presence of a unique region, designated pX. The pX region encodes several accessory genes, which control viral replication and the proliferation of infected cells (Matsuoka 2005).

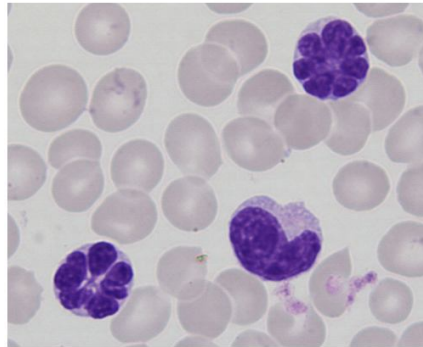


Fig. 2. Typical "flower cell", Morphological findings of typical ATL cells, leukemic cells with multilobulated nuclei was Shawn.

Several molecular biologic studies have reported that various cellular dysfunctions induced by viral genes (eg, tax and HBZ), genetic and epigenetic alterations, and the host immune system may be involved in the leukemogenesis of ATL. Clinical and epidemiologic studies have also reported a variety of possible risk factors for ATL, including vertical transmission of HTLV-1 infection, male gender, a long latent period, increased leukocyte counts or abnormal lymphocyte counts, and higher levels of anti-HTLV-1 antibody titers and soluble interleukin-2 receptor. However, there are no clear determinants that separate those who develop ATL from those who remain healthy carriers. Recently, HTLV-1 proviral load levels have been evaluated as important predictors of development of ATL and HAM/TSP. Some cross-sectional studies showed that HTLV-1 proviral load levels were higher in ATL and HAM/TSP compared with asymptomatic HTLV-1 carriers. In conclusion, the cohort study of 1218 asymptomatic HTLV-1 carriers provided detailed distributions for HTLV-1 proviral loads regarding the host-specific characteristics and the associations with the development of ATL. A higher proviral load levels (especially > 4 copies/100 PBMCs), advanced age, family history of ATL, and having the first opportunity to learn of HTLV-1 infection during treatment of other diseases are independent risk factors for progression from carrier status to ATL.

Further large-scale epidemiologic studies are needed to clearly identify the determinants of ATL for early detection and rapid cure for HTLV-1-associated diseases (Silva et al. 2007). Genetic and immunological factors in the host are the principal determinants of the emergence of associated diseases (Romanelli, Caramelli and Proietti 2010).

7.1 Etiology of ATL

The most important aspect of the new retroviral isolation was not just novelty but an etiology for a human leukemia. However, etiological proof for a human disease is generally not easy unless an animal model is available. The most critical question thereafter was whether 'close association of HTLV-1 with ATL' reflects its causative role or whether the virus was just a passenger. The nature of provirus integration of the retroviruses provided a critical tool for the discrimination. The retroviral genomes are generally reverse-transcribed into provirus DNA, and the proviral genomes are integrated into host cell DNA at random sites. Since a tumor originates from unlimited expansion of a single malignant cell, the site for the proviral integration into tumor cells would be uniform in individuals if the retroviral infection plays a causative role; but if the virus fortuitously infects leukemic cells, then the integration sites would be random. Southern blot analysis of patients' leukemic cell DNA clearly indicated clonal integration in each patient revealing two distinct bands with cellular flanking sequences. This finding clearly supported the virus playing a causative role in ATL. Virtually all ATL cases were clonally infected leukemic cells; therefore, the conclusion for a 'causative role' became generally accepted. As controls, the sites for the integration in viral carriers are random except only in a few cases which show clonal integration with higher viral burden (Yoshida 2005).

7.2 Pathogenesis of HTLV-1 infection

ATL cells are derived from activated helper T-lymphocytes, which play central roles in the immune system by elaborating cytokines and expressing immunoregulatory molecules. ATL cells are known to retain such features and this cytokine production or surface molecule expression may modify the pathogenesis. ATL is well known to infiltrate various organs and tissues, such as the skin, lungs, liver, gastrointestinal tract, central nervous system and bone (Takatsuki 1995). This infiltrative tendency of leukemic cells is possibly attributable to the expressions of various surface molecules, such as chemokine receptors and adhesion molecules. Skin-homing memory T-cells uniformly expresses CCR4, and its ligands are thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC). CCR4 is expressed on most ATL cells. In addition, TARC and MDC are expressed in skin lesions in ATL patients. Thus, CCR4 expression should be implicated in the skin infiltration (Yoshie et al. 2002). On the other hand, CCR7 expression is associated with lymph node involvement (Hasegawa et al. 2000). OX40 is a member of the tumor necrosis factor family, and was reported to be expressed on ATL cells (Higashimura et al. 1996). It was also identified as a gene associated with the adhesion of ATL cells to endothelial cells by a functional cloning system using a monoclonal antibody that inhibited the attachment of ATL cells (Imura et al. 1996). Thus, OX40 is also implicated in the cell adhesion and infiltration of ATL cells. Thus, ATL cells express various molecules that can modify their phenotypic properties, thereby modifying the clinical disease manifestation, and facilitating the survival of ATL cells (Matsuoka 2003). Hypercalcemia is frequently complicated in patients with acute ATL (more than 70% during the whole clinical course)

(Kiyokawa et al. 1987). In hypercalcemic patients, the number of osteoclasts increases in the bone. RANK ligand, which is expressed on osteoblasts, and M-CSF act synergistically on hematopoietic precursor cells, and induce the differentiation into osteoclasts (Arai et al. 1999). ATL cells from hypercalcemic ATL patients express RANK ligand, and induced the differentiation of hematopoietic stem cells into osteoclasts when ATL cells were co-cultured with hematopoietic stem cells (Nosaka et al. 2002). In addition, the serum level of parathyroid hormone-related peptide (PTH-rP) is also elevated in most of hypercalcemic ATL patients. PTH-rP indirectly increases the number of osteoclasts, as well as activating them (Watanabe et al. 1990), which is also implicated in mechanisms of hypercalcemia.

7.3 Role of Tax in HTLV-1 induced oncogenesis

Tax, a transactivator protein, triggers a plethora of events like cell signaling, cell cycle regulation and interference with checkpoint control and inhibition of DNA repair. Tax is expressed from a doubly spliced mRNA transcript. Although Tax shares the same mRNA transcript with Rex, translation of Tax is favored over Rex due to a stronger Kozak sequence. Tax made in the cytoplasm is translocated into the nucleus, where it binds to its response element and activates viral LTR-mediated transcription. (Boxus and Willems 2009).

The tax gene plays central roles in viral gene transcription, viral replication and the proliferation of HTLV-1-infected cells. Tax enhances viral gene transcription from the 5'-LTR via interaction with cyclic AMP responsive element binding protein (CREB). Tax also interacts with cellular factors and activates transcriptional pathways, such as NF- κ B, AP-1 and SRF (Yoshida 2001). For example, activation of NF- κ B induces the transcription of various cytokines and their receptor genes, as well as anti-apoptotic genes such as bcl-*x*L and survivin (Tsukahara et al. 1999). The activation of NF- κ B has been demonstrated to be critical for tumorigenesis both *in vitro* and *in vivo* (Mori et al. 1999). On the other hand, Tax variant without activation of NF- κ B has also been reported to immortalize primary T-lymphocytes *in vitro*, suggesting that mechanisms of immortalization are complex. In addition to NF- κ B, activation of other transcriptional pathways such as CREB by Tax should be implicated in the immortalization and leukemogenesis. Tax also interferes with the functions of p53, p16 and MAD1 (Ariumi et al. 2000). These interactions enable HTLV-1-infected cells to escape from apoptosis, and also induce genetic instability. Although inactivation of p53 function by Tax is reported to be mediated by p300/CBP or NF- κ B activation (Pise-Masison et al. 2000), Tax can still repress p53's activity in spite of loss of p300/CBP binding or in cells lacking NF- κ B activation (Miyazato et al. 2005), indicating the mechanism of p53 inactivation by Tax needs further investigation. Although Tax promotes the proliferation of infected cells, it is also the major target of cytotoxic T-lymphocytes (CTLs) *in vivo*. Moreover, excess expression of Tax protein is considered to be harmful to infected cells. Therefore, HTLV-1 has redundant mechanisms to suppress Tax expression. Rex binds to Rex-responsive element (RxRE) in the U3 and R regions of the 3'-LTR, and enhances the transport of the unspliced gag/pol and the singly spliced env transcripts. By this mechanism, double-spliced tax/rex mRNA decreases, resulting in suppressed expression of Tax (Inoue, Yoshida and Seiki 1987). Additionally, p30 binds to tax/rex transcripts, and retains them in the nucleus. The HBZ gene is encoded by the complementary strand of HTLV-1, and contains a leucine zipper domain. HBZ directly interacts with c-Jun or JunB (Basbous et al. 2003), or enhances their degradation, resulting in the suppression of Tax-mediated viral transcription from the LTR. Transforming growth factor- β (TGF- β) is an inhibitory cytokine that plays important roles in development, the

immune system and oncogenesis. Since TGF- β generally suppresses the growth of tumor cells, most tumor cells acquire escape mechanisms that inhibit TGF- β signaling, including mutations in its receptor and in the Smad molecules that transduce the signal from the receptor. Tax has also been reported to inhibit TGF- β signaling by binding to Smad2, 3 and 4 or CBP/p300 (Mori et al. 2001). Inhibition of TGF- β signaling enables HTLV-1-infected cells to escape TGF- β -mediated growth inhibition. ATL cells have been reported to show remarkable chromosomal abnormalities (Sanada et al. 1986), implicated in the disease progression. Tax has been reported to interact with the checkpoint protein MAD1, which forms a complex with MAD2 and controls the mitotic checkpoint. This functional hindrance of MAD1 by Tax protein causes chromosomal instability, suggesting the involvement of this mechanism in oncogenesis. Recently, Tax has been reported to interact with Cdc20 and activate Cdc20-associated anaphase-promoting complex, an E3 ubiquitin ligase that controls the metaphase-to-anaphase transition, thereby resulting in mitotic abnormalities (Liu et al. 2005). In contrast to HTLV-1, HTLV-2 promotes the proliferation of CD8-positive T-lymphocytes *in vivo*. Although it was first discovered in a patient with variant hairy cell leukemia, HTLV-2 is less likely to have oncogenic properties since there is no obvious association between HTLV-2 infections and cancers. Regardless of the homology of their tax sequences, the oncogenic potential of Tax1 (HTLV-1 Tax) is more prominent than that of Tax2 (HTLV-2 Tax). The most striking difference is that Tax2 lacks the binding motif at C-terminal end to PDZ domain proteins, while Tax 1 retains it. When the PDZ domain of Tax1 is added to Tax2, the latter acquires oncogenic properties in the rat fibroblast cell line Rat-1, indicating that this domain is responsible for the transforming activity of HTLV-1. To understand the pleiotropic actions of Tax protein more clearly, transcriptome analyses are essential. The transcriptional changes induced by Tax expression have been studied using DNA microarrays, which revealed that Tax upregulated the expression of the mixed-lineage kinase MLK3. MLK3 is involved in NF- κ B activation by Tax as well as NIK and MEKK1. In addition to transcriptional changes, Tax is also well known to interact with cellular proteins and impair or alter their functions. For example, proteomic analyses of Tax-associated complexes showed that Tax could interact with cellular proteins, including the active forms of small GTPases, such as Cdc42, RhoA and Rac1, which should be implicated in the migration, invasion and adhesion of T-cells, as well as in the activation of the Jun-kinase (JNK) pathway (figure 3) (Matsuoka 2005).

Tax1 upregulates the expression of genes encoding cytokines, chemokines, cell surface ligands, and their receptors, in an NF- κ B, AP-1, CREB/ATF and/or NFAT dependent manner. They include IL-2 receptor (IL-2R) α -chain, IL-9, IL-13, IL-15/IL-15R, IL-21/IL-21R, IL-8, CCL2, CCL5, CCL22, CCR9, CXCR7, CD40, OX40/OX40L, and 4-1BB/4-1BBL. Among these, the IL-2R α -chain is crucially important for T-cell immortalization by Tax, since the immortalized cells are dependent on IL-2 for their growth.

ATL cells are well known to infiltrate into various organs or tissues, frequently invading skin or lymphoid tissues. Analysis of chemokine receptor expression revealed that CCR4 was frequently expressed on HTLV-1-transformed cell lines and fresh ATL cells. CCR4-positive T lymphocytes contain skin-seeking memory T cells, accounting for frequent infiltration of ATL cells into skin. On the other hand, expression of CCR7 was reported to be associated with the involvement of lymphoid tissues, and lymph node enlargement. A subtraction strategy between ATL cell lines and activated T cells identified I-309 as a secreted chemokine from ATL cells, and I-309 expression was remarkably enhanced in ATL cell lines. I-309 showed anti apoptotic effect via its receptor CCR8, invoking that an autocrine mechanism via I-309/CCR8 allowed ATL cells to survive *in vivo* (Matsuoka 2003).

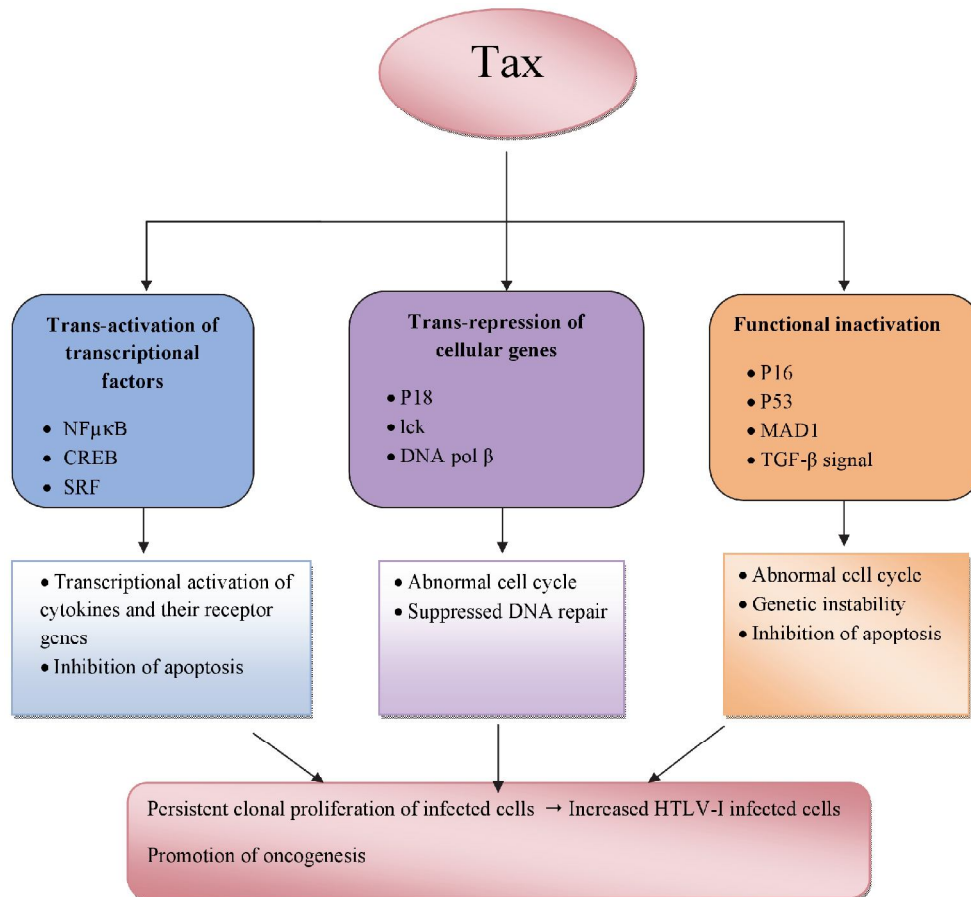


Fig. 3. Pleiotropic actions of Tax. Pleiotropic actions of Tax proteins are summarized

7.4 Inactivation of Tax expression in ATL cells

As mentioned above, Tax expression confers advantages and disadvantages on HTLV-1-infected cells. Although the proliferation of infected cells is promoted by Tax expression, CTLs attack the Tax-expressing cells since Tax is their major target. In HTLV-1-infected cells, Rex, p30 and HBZ suppress Tax expression. On the other hand, loss of Tax expression is frequently observed in leukemic cells. Three mechanisms have been identified for inactivation of Tax expression: 1) genetic changes of the tax gene (non-sense mutations, deletions or insertions); 2) DNA methylation of the 5'-LTR; and 3) deletion of the 5'-LTR. Among fresh leukemic cells isolated from ATL patients, about 60% of cases do not express the tax gene transcript. Interestingly, ATL cells with genetic changes of the tax gene expressed its transcripts, suggesting that ATL cells do not silence the transcription when the tax gene is abortive. Loss of Tax expression gives ATL cells advantage for their survival since they can escape from CTLs (Figure 4) (Matsuoka 2005).

Some ATL cells can proliferate without functional Tax protein, suggesting that somatic (genetic and epigenetic) alterations cause transcriptional or functional changes to the host

genes. The mutation rate of the p53 gene in ATL cells has been reported to be 36% (4/11) and 30% (3/10) (Nishimura et al. 1995). The p16 gene is an inhibitor of cyclin-dependent kinase 4/6, and blocks the cell cycle. Deletion and aberrant methylation of the p16 gene has also been reported in ATL cells. In addition, genetic changes in the p27KIP1, RB1/p105 and RB2/p130 genes have been reported in ATL, although they are relatively rare: 2/42 (4.8%) for the p27KIP1 gene; 2/40 (5%) for the RB1/p105 gene; and 1/41 (2.4%) for the RB2/p130 gene) (Morosetti et al. 1995). The fact that higher frequencies of genetic changes in these tumor suppressor genes are observed among aggressive forms of ATL suggests that such genetic changes are implicated in disease progression. Fas antigen was the first identified death receptor. It transduces the death signal by binding of its ligand, Fas ligand (FasL). ATL cells highly express Fas antigen on their cell surface (Nagata 1999), and are highly susceptible to death signals mediated by agonistic antibodies to Fas antigen, such as CH-11. Genetic changes of Fas gene in ATL cells, which confer resistance to the Fas-mediated signal, have been reported (Tamiya et al. 1998). Normal activated T-lymphocytes express FasL as well as Fas antigen. Apoptosis induced by autocrine mechanisms is designated activation-induced cell death (AICD) and this controls the

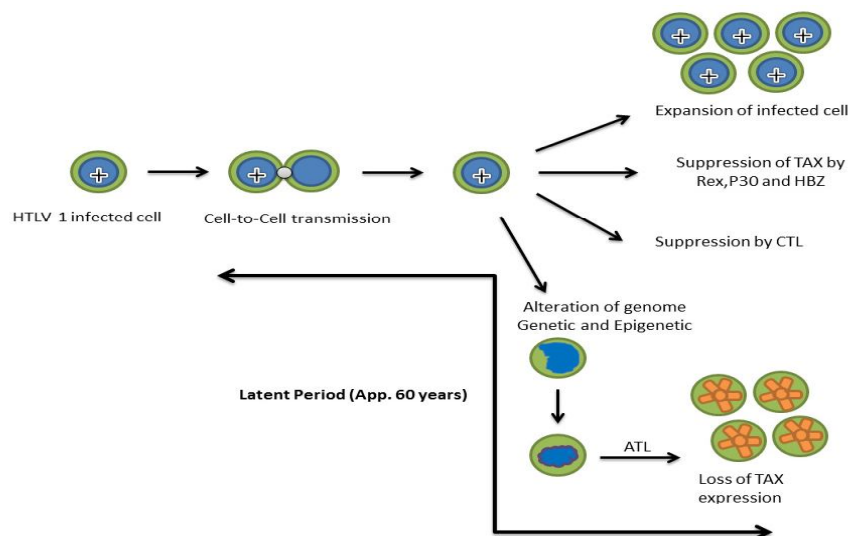


Fig. 4. Natural course from the infection of HTLV-I to onset of ATL

immune response (Krueger et al. 2003). Although ATL cells express Fas antigen, they do not produce FasL, thereby enabling ATL cells to escape from AICD. Attempts to isolate hypermethylated genes from ATL cells identified the EGR3 gene as a hypermethylated gene compared to PBMCs from carriers (Yasunaga et al. 2004). EGR3 is a transcriptional factor with a zinc finger domain, which is essential for transcription of the FasL gene. The finding that EGR3 gene transcription is silenced in ATL cells could account for the loss of FasL expression, and the escape of ATL cells from AICD. Thus, alterations of the Fas (genetic) and EGR3 (epigenetic) genes are examples of ATL cell evolution *in vivo*. Disordered DNA

methylation has been identified in the genome of ATL cells compared with that of PBMCs from carriers: hypomethylation is associated with aberrant expression of the MELIS gene (Yoshida et al. 2004), while hypermethylation silences transcription of the p16 (Nosaka et al. 2000), EGR3 and KLF4 genes as well as many others. It is reasonable to consider that other currently unidentified genes are involved in such alterations of the genome in ATL cells, and play roles in leukemogenesis. Transcriptome analyses using DNA microarrays have revealed transcriptional changes that are specific to ATL cells. Among 192 up-regulated genes, the expressions of the tumor suppressor in lung cancer 1 (TSLC1), caveolin 1 and prostaglandin D2 synthase genes were increased more than 30-fold in fresh ATL cells compared with normal CD4⁺ and CD4⁺, CD45RO⁺ T-cells (Sasaki et al. 2005). TSLC1 is a cell adhesion molecule that acts as a tumor suppressor in lung cancer. Although TSLC1 is not expressed on normal T- lymphocytes, all acute ATL cells show ectopic TSLC1 expression. Enforced expression of TSLC1 enhances both the self-aggregation and adhesion abilities to vascular endothelial cells in ATL cells. Thus, TSLC1 expression is implicated in the adhesion or infiltration of ATL cells. A retrovirus cDNA library screening from ATL cells, a gene with oncogenic potency was identified in NIH3T3 cells, and designated the Tgat gene. Ectopic expression of the Tgat gene is observed in aggressive forms of ATL, and *in vitro* experiments showed that its expression is associated with an invasive phenotype (Matsuoka 2005).

7.5 Role of HBZ in HTLV-1-induced oncogenesis

The pathogenesis of ATL involves four stages: infection, polyclonal proliferation, clinical latency and tumorigenesis. HTLV-1 induced ATL after a long latent period. Previous studies suggested the significance of the tax gene. However, Tax is not expressed in approximately 60% of ATL cases by three mechanisms: 1) deletion of 5' long terminal repeat (LTR), 2) DNA methylation of 5 LTR, and 3) genetic changes of the tax gene. Recent studies, demonstrated that the HTLV-1 basic leucine zipper factor (HBZ) encoded by the virus in an antisense orientation may play a critical role in the malignant proliferation of ATL cells. The expression of HBZ gene is detected in all ATL cases, and this is due to the usage of the promoter in the 3' LTR of HTLV-1 gene which is not inactivated in the ATL cells. HBZ interacts with various host factors, including c-Jun, JunB, JunD, and p65. Thus, HBZ modulates cellular signal pathways in addition to promoted proliferation. These findings indicate that HBZ is an essential viral gene for oncogenesis by HTLV-1 (Boxus and Willems 2009).

Short hairpin RNA mediated knockdowns of HBZ expression in both ATL and HTLV-1 transformed cell lines reduce their proliferation. Moreover, transgenic mice expressing HBZ under the control of the CD4 promoter/enhancer display increased numbers of CD4-positive T-cells in the spleen, and augmented proliferation of thymocytes after anti-CD3 stimulation. Thus, these findings indicate that HBZ has a growth promoting activity, and could be involved in the malignant proliferation of ATL cells *in vivo*, although the precise molecular mechanism for these findings is still unclear. HTLV-2 also encodes a HBZ like protein, designated as the antisense protein of HTLV-2 (APH-2). Interestingly, unlike HBZ, APH-2 does not have a leucine zipper motif which is essential for various HBZ functions. Thus, it is important to study whether the HTLV-2 APH-2 protein has a growth promoting activity in T-cells like HBZ in order to understand better how these two viruses show distinct pathogenicities (Higuchi and Fujii 2009).

Both the HBZ and Tax genes are found in the genome of the simian T-cell leukemia virus type 1 (STLV-1), which shares a common ancestor with HTLV-1, indicating that HBZ has not been recently acquired; that is once the virus adapted to humans (STLV-1 and HTLV-1 are considered to have diverged around 50 000 years ago. it did not tolerate genetic drift resulting in its silencing. In the HTLV-2 genome, a human retrovirus related to HTLV-1, Tax also exists but, surprisingly, HTLV-2 lacks the HBZ-ORF. Moreover, in contrast to HTLV-1 and STLV-1, which both cause lymphoid malignancy in the host, no association between HTLV-2 infection and cancer has been yet evidenced. There has been only one reported case of a patient carrying HTLV-2 who developed a variant of hairy cell leukemia (Mesnard, Barbeau and Devaux 2006).

8. ATL treatment: Current state and new strategies

In spite of intensive chemotherapies, the prognosis of ATL patients has not improved. The median survival time of acute or lymphoma-type ATL was reported to be *13 months* with the most intensive chemotherapy. Such a poor prognosis might be due to: 1) the resistance of ATL cells to anti-cancer drugs; and 2) the immunodeficient state and complicated opportunistic infections. One mechanism of resistance to anti-cancer drugs is the activated NF- κ B pathway in ATL cells (Mori et al. 1999), which increases the transcription of anti-apoptotic genes such as bcl-xL and survivin.

A proteasome inhibitor, Bortezomib, is currently used for the treatment of multiple myeloma. One of its mechanisms is suppression of the NF- κ B pathway by inhibiting the proteasomal degradation of I κ B protein. Several groups have shown that Bortezomib is effective against ATL cells both *in vitro* and *in vivo* (Mitra-Kaushik et al. 2004). The sensitivity to Bortezomib is well correlated with the extent of NF- κ B activation. Depsipeptide is a histone deacetylase inhibitor, and a clinical trial on its use in cutaneous T-cell lymphoma has commenced. This drug also inhibits the activation of NF- κ B and AP-1 in ATL cells, and it induces apoptosis (Mori et al. 2004).

An alternative approach to the therapy of ATL is to target cell-surface markers on the malignant cells with monoclonal antibodies. Anti- CD25 (anti-Tac) monoclonal antibody, which was first administered to patients with ATL in the late 1980s, was reported to be effective in some patients, with a complete response in 2 of 19 patients and a partial response in 4 of 19 patients (Waldmann et al. 1993). Another antibody, anti- CD52 monoclonal antibody (Campath-1H), is being evaluated in a phase II clinical trial by the National Institutes of Health (Protocol 03-C-0194). Humanized anti-CD2 antibody (MEDI-507) has also been shown to be effective *in vivo* (Zhang et al. 2003). Bortezomib effect could be enhanced by combined use of anti-CD25 antibody (Tan and Waldmann 2002). During chemotherapy for ATL, chemotherapeutic agents worsens the immunodeficient state of ATL patients. Antibody therapy against ATL cells has advantages due to its decreased adverse effects.

As described above, most ATL cells express CCR4 antigen on their surfaces, and a humanized antibody against CCR4 is being developed as an anti-ATL agent (Ishida et al. 2004). Advances in the treatment of ATL were brought about by allogeneic bone marrow or stem cell transplantation (Borg et al. 1996; Yamada et al. 2001). Absence of graft-versus-host disease (GVHD) was linked with relapse of ATL, suggesting that GVHD or graft-versus-ATL may be implicated in the clinical effects of allogeneic stem cell transplantation (Borg et al. 1996). Furthermore, 16 patients with ATL, who were over 50 years of age, were treated

with allogeneic stem cell transplantation with reduced conditioning intensity (RIST) from HLA-matched sibling donors (Okamura et al. 2005). Among 9 patients in whom ATL relapsed after transplantation, 3 achieved a second complete remission after rapid discontinuation of cyclosporine A. This finding strongly suggests the presence of a graft-versus-ATL effect in these patients. In addition, Tax peptide-recognizing cells were detected by a tetramer assay (HLA-A2/Tax 11-19 or HLA-A24/Tax 301-309) in patients after allogeneic stem cell transplantation (Harashima et al. 2004). In 8 patients, the provirus became undetectable by real-time PCR. Among these, 2 patients who received grafts from HTLV-1-positive donors also became provirus-negative after RIST. Since the provirus load is relatively constant in HTLV-1-infected individuals (Etoh et al. 1999), this finding indicates an enhanced immune response against HTLV-1 after RIST, which suppresses the provirus load. This may account for the effectiveness of allogeneic stem cell transplantation to ATL. However, Tax expression is frequently lost in ATL cells as described above. Many questions arise, such as whether the tax gene status is correlated with the effect of allogeneic stem cell transplantation, and whether the effectiveness of the anti-HTLV-1 immune response is against leukemic cells or non-leukemic HTLV-1-infected cells. Nevertheless, these data suggest that potentiation of the immune response against viral proteins such as Tax may be an attractive way to treat ATL patients. Such strategies may enable preventive treatment of high-risk HTLV-1 carriers, such as those with familial ATL history, predisposing genetic factors to ATL, a higher provirus load, etc (Matsuoka 2005).

The leukemic phase of ATL tends to spare the bone marrow; accentuated anemia and thrombocytopenia are not observed. White blood cell counts are always elevated and can be as high as 100,000/mm³. Heightened leukocyte counts and elevated lactate dehydrogenase (LDH) and calcium levels are markers of worse prognosis. Atypical lymphocytes that are pleomorphic and lobulated and have significant nuclear abnormalities (flower cells) are found in peripheral blood. If left untreated it is rapidly fatal, with death caused by pulmonary complications, opportunistic infections, sepsis and uncontrolled hypercalcemia. The chronic and indolent forms of ATL are less common, but after a number of years they will evolve into the acute form. Treatment of the indolent and chronic forms can be postponed until they evolve into the acute form; despite a less aggressive clinical course, prognosis for survival over the long term is poor. Some studies, with small patient samples and short follow-up periods, have demonstrated a satisfactory response and moderate toxicity using zidovudine in combination with interferon alpha, and both of these in combination with arsenic. In the more aggressive acute and lymphomatous forms, treatment should be started as early as possible using, CHOP chemotherapy regimens (cyclophosphamide, doxorubicin, vincristine and prednisolone). More powerful regimens such as VCAP (vincristine, cyclophosphamide, doxorubicin and prednisolone) or AMP (doxorubicin, ranimustine and prednisolone), offer a better response and prognosis, but mortality is higher. Other treatment options described in the literature include allogeneic stem cell transplant, inhibition of the NF-kappa B protein and monoclonal antibodies (Romanelli, Caramelli and Proietti 2010).

Regardless of the extensive progress in virology, immunology and molecular biology of ATL and HTLV-1, the prognosis of patients with ATL remains poor. ATL is generally treated with aggressive combination chemotherapy, but long-term success has been less than 10%. The acute form, with hypercalcemia, high LDH levels and an elevated white blood cell count shows a particularly poor prognosis. Although G-CSF supported

combination chemotherapy with eight drugs improved the survival (mean survival time 13 months), the prognosis of aggressive ATL remains poor with deaths usually being the result of severe infection or hypercalcemia, often associated with drug resistance. After successful allogeneic bone marrow transplantation (alloBMT) for a patient with ATL was reported, more patients with ATL were treated with alloBMT. The low risk of relapse in cases with graft-versus-host disease, suggested that graft-versus-leukemia was effective against ATL cells. CTLs attack ATL cells via Fas ligand, perforin or granzyme. These results are consistent with the finding that ATL cells are highly susceptible to the signal via Fas antigen. Thus, the signal through Fas antigen might be a good target in therapy against ATL (Matsuoka 2003).

In a phase II study, combination of zidovudine and interferon-alpha presented promising results. Chronic ATL has a relatively better out-come, but poor long-term survival is noted when patients are managed with a watchful-waiting policy or with chemotherapy. In ATL cell lines, arsenic trioxide shuts off constitutive NF- κ B activation and potentiates interferon-alpha apoptotic effects through proteasomal degradation of Tax. In conclusion, treatment of chronic ATL with arsenic, interferon- alpha, and zidovudine is feasible and exhibits an impressive response rate with moderate toxicity. Viral replication (AZT) and Tax degradation (As/IFN) may eradicate the disease through this treatment. These clinical results strengthen the concept of oncogene-targeted cancer therapy (Kchour et al. 2009).

Key Words: HTLV-1, ATL, Leukemia, Molecular pathogenesis, Diagnosis, Novel treatments, Oncogenesis, Mutation, Arsenic, Interferon-alpha, Zidovudine

9. References

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