Human T-cell leukemia virus

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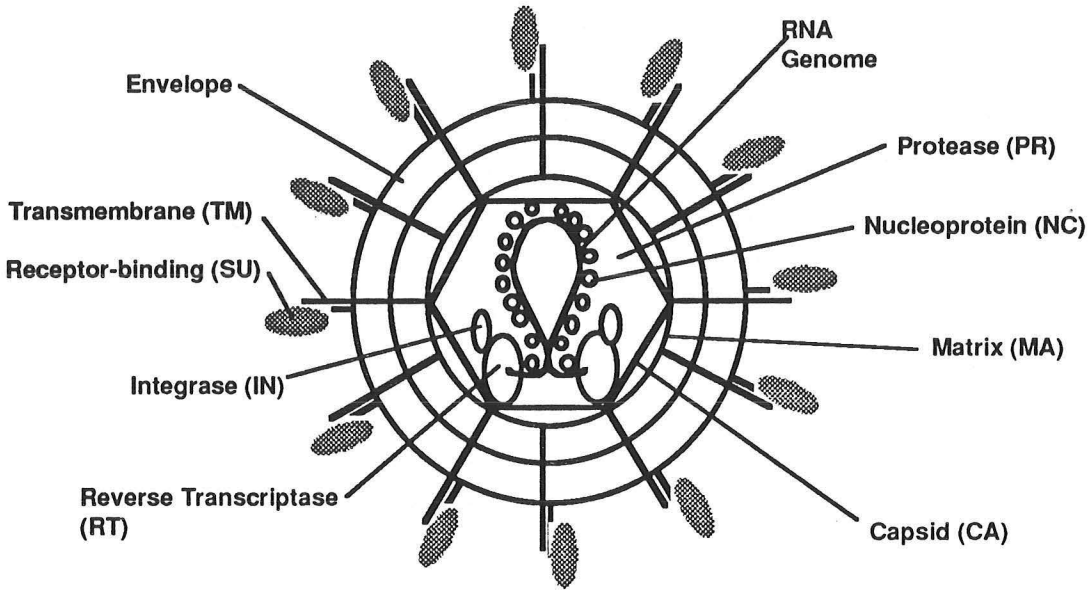
Biology of Animal Retroviruses

Retroviruses comprise a large family of viruses that are associated with many diseases including either rapid or long-latency malignancies, wasting syndromes, neurological disorders, and immunodeficiencies, as well as lifelong viremia without effects on the host. These viruses have been the subject of intense scientific investigation for the last decade. This interest stems from several facts. First retroviruses are the causative agents of human diseases including AIDS and adult human T-cell leukemia (ATL). Second their ability to alter their genomes by mutation and recombination has been important in understanding the means viruses adopt to evade immune surveillance. Third due to the relationship between the virus and the host, a great deal has been learned about the regulation of host cell gene expression. Finally due to the unique replication cycle of retroviruses, the ability to acquire and alter host-derived DNA sequences or either activate or inactivate cellular genes has been crucial in our identification and understanding of cellular oncogenes. Studies of retroviruses are critical in understanding many different aspects of human biology. A detailed review of retrovirus biology is presented by Coffin (Virology, Eds Fields and Knipe, 1990).

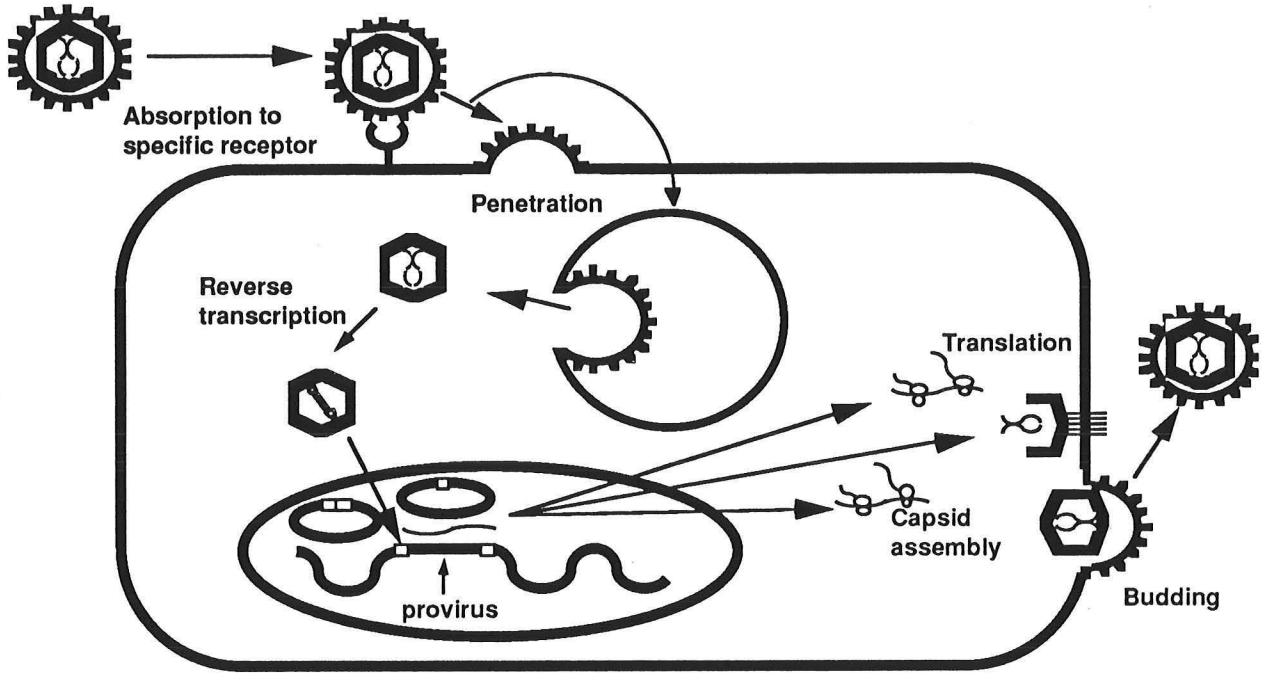
All retrovirus isolates are quite similar in virion structure, genome organization and mode of replication. The virion is enveloped and is approximately 100 nm in diameter. Its surface is covered by proteins encoded by the envelope (env) gene. The internal nucleocapsid or core is a spherical structure made up of several products of the gag gene. The core is also made up of several proteins that have catalytic roles during replication. They include the reverse transcriptase which converts the genetic information from single-strand RNA to double-strand DNA and the integrase which is necessary for covalently joining viruses to cell DNA to form the provirus. At both termini of the provirus are two direct repeats known as long terminal repeats, which function in activating proviral gene expression and polyadenylation of viral RNAs. The genome consists of two (usually identical) molecules of single-strand RNA, ranging from about 7 to 10 kb in length. The order of the genes encoding structural proteins is invariably *gag-pol-env*. A number of other genes involved in regulation of viral expression are present in some viral groups.

The Retrovirus Virion



The viral replication cycle proceeds in two phases. The first phase includes (a) entry of the virion core into the cytoplasm, (b) synthesis of double-strand DNA using the single-strand RNA genome as template, (c) transfer of the viral DNA to the nucleus, and (d) integration of the DNA into the host genome. These steps are mediated by proteins found within the virion and proceed in the absence of viral gene expression. The second phase includes the synthesis and production of viral RNAs and proteins in conjunction with host cell proteins such as RNA polymerase and additional cellular factors involved in both transcriptional and translational control. Virion assembly proceeds by encapsidation of the genome by gag and gag-pol fusion proteins, processing of these to the finished products, association of the nucleocapsids with the cell membrane, and release of the virion by budding.

Overview of Retrovirus Replication



Retroviruses have been traditionally divided into three subfamilies based primarily on pathogenicity rather than on genome relationships. They are known as Oncovirinae, which includes human T-cell leukemia virus, Lentivirinae, which includes the human immunodeficiency virus, and Spumavirinae which includes the human foamy viruses, Viruses are further divided according to the following: (a) virion structure (types A to D), (b) utilization of particular cell receptors, (c) whether endogenous (passed from parent to offspring as a proviruses integrated into the germline) or exogenous, (d) presence or absence of an oncogene, and (e) other pathogenic properties. When nucleotide sequence relationship and genome structure are used as criteria, seven groups of viruses comprising all well-analyzed isolates can be recognized.

DISCOVERY OF HTLV-I

In the 1970s several investigators described patients with both chronic and acute T-cell lymphoproliferative disorders affecting adults born in southwestern Japan (Yodoi et al. 1974a,b; Uchiyama et al. 1977). The acute T-cell lymphoproliferative disorder was characterized by the presence of

pleomorphic neoplastic cells with the membrane markers of mature T lymphocytes. Other findings common at presentation include lymphadenopathy, hepatomegaly, splenomegaly, cutaneous infiltration with neoplastic T cells, hypercalcemia (with or without lytic bone lesions), and interstitial pulmonary infiltrates. Characteristically, mediastinal tumors were not involved. The geographic distribution of the cases led these investigators to conclude that this entity known as adult T-cell leukemia (ATL) represented a new category of T-cell malignancy (Uchiyama et al. 1977).

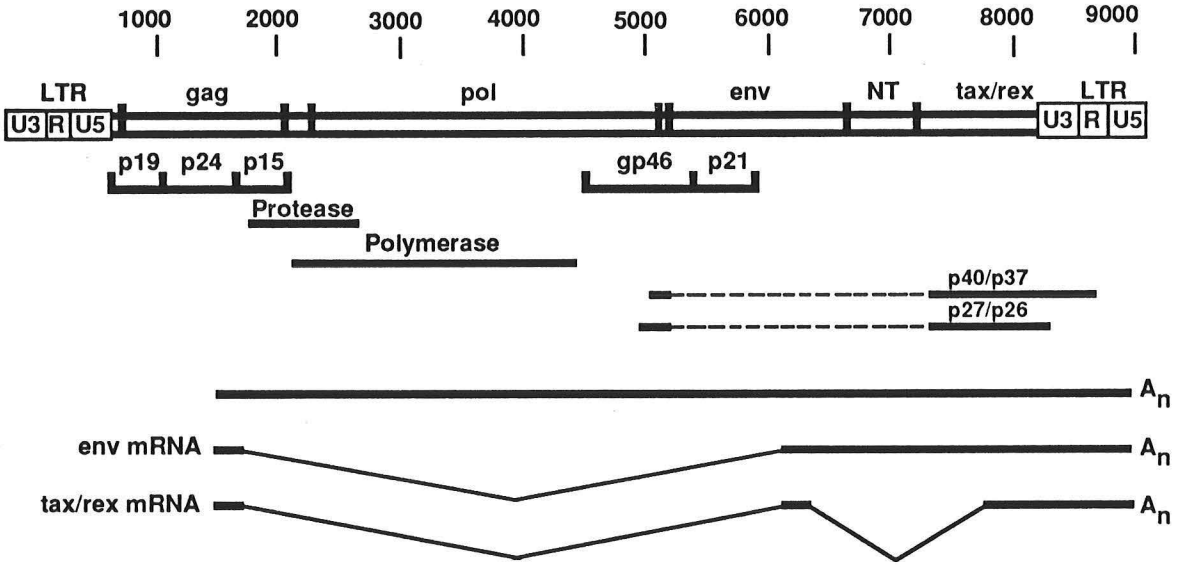
At about the same time Gallo and associates were studying American patients who originally had been diagnosed as having mycosis fungoides/Sezary syndrome. In 1978, these researchers identified and isolated from these patients the first human tumor virus and named their isolate human T-cell leukemia/lymphoma virus (HTLV-I) (Poiesz et al. 1980; Poiesz et al. 1981; Kalyanaraman et al. 1981). HTLV-I was not closely related to any known retroviruses in antigenicity or nucleic-acid sequence homology. This finding led to the discovery that a common retrovirus was involved in both these disease states (Hinuma et al. 1981; Yoshida et al. 1982; Watanabe et al. 1984). Subsequently HTLV-I was associated with a variety of clinical manifestations seen in patients found worldwide. Another virus was subsequently discovered by Gallo and his colleagues which was related antigenically and at the nucleic acid level with HTLV-I (Kalyanaraman et al., 1982). This isolate which was sometimes associated with T-cell neoplasms was designated HTLV-II.

MOLECULAR BIOLOGY OF HTLV

Both HTLV-I and HTLV-II have been molecularly cloned and sequenced, permitting intensive investigation of their molecular biology (reviewed in Rosenblatt et al. 1988). The size and structure of both HTLV-I and HTLV-II resemble that of other oncogenic replication competent retroviruses, with the exception of the presence of unique 3' sequences, termed the X region (reviewed in Rosenblatt et al. 1988). The HTLV-I and HTLV-II genomes measure 9.03 and 8.95 kbp, respectively. The HTLV genome is bounded by a long terminal repeat (LTR) that contains sequences required for viral integration, reverse transcription, and the regulation of proviral expression. Only 35% of nucleic acid sequences in the LTR are conserved between HTLV-I and HTLV-II; however, specific

sequences thought to be involved in the regulation of proviral gene expression are highly conserved.

Organization of the HTLV Genome



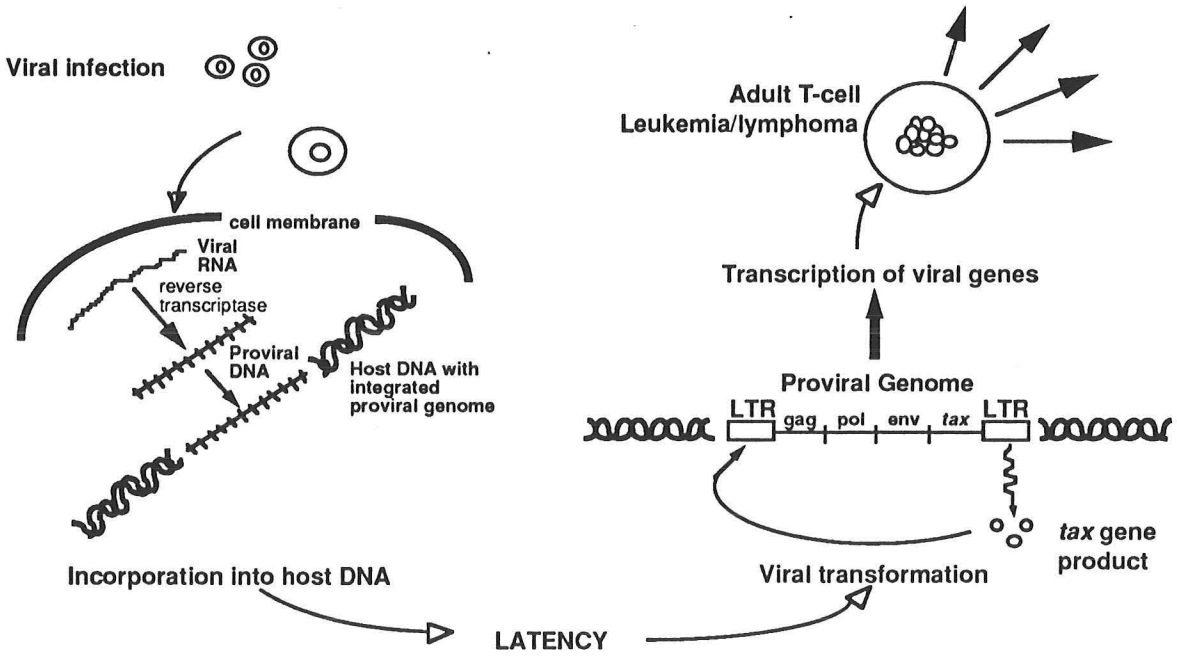
The HTLV gag gene, which encodes the viral group specific antigens, encodes a precursor protein that is processed into three separate products estimated to be 9, 15, and 26 KDa. The 3' end of both the HTLV-I and HTLV-II *gag* genes overlaps an open reading frame for a putative viral protease believed to be necessary for cleavage of the precursor *gag* protein. The HTLV *pol* gene overlaps the protease gene at its 5' end and extends into *env* gene sequences at its 3' end. In addition to reverse transcriptase, the *pol* gene contains sequences that encode an RNAse H and an intergrase. These proteins facilitate the synthesis of a viral DNA intermediate, and its subsequent integration into the host genome.

The HTLV env gene encodes the retroviral envelope precursor protein whose size is estimated to be 67 KDa. This protein is subsequently cleaved and glycosylated. The env-encoded glycoproteins are thought to interact with specific antigenic determinants on the surface of cells, facilitating viral adsorption. The specific target antigens for this interaction have not been identified. Though both HTLV-I and HTLV-II transform only $CD4^+$ lymphocytes, their ability to infect a variety of other cell lines indicates that cell entry is not mediated by the $CD4^+$ receptor as with HIV. Recent studies with both intact and heat inactivated

virions suggest that env proteins themselves may play a central role in the induction of T cell proliferation (Gazzolo and DucDoden, 1987).

The related oncogenic retroviruses, HTLV-I, HTLV-II and bovine leukemia virus, all contain unique X region sequences located between the env gene and the 3' LTR. The X region does not exhibit homology to any known cellular proto-oncogene, and harbors at least two distinct genes that are encoded on the same subgenomic mRNA species from different but overlapping reading frames. The first gene identified in the X region was known as *tax* which encodes a protein of approximately 40 KDa. *Tax* is essential to activate HTLV-I gene expression and it can also activate specific cellular genes (Carr et al. 1985; Felber et al. 1985; Greene et al. 1986; Kronke et al. 1988; Siekevitz et al. 1987). *Tax* is localized to the cell nucleus and has a short half-life estimated at 120 minutes consistent with that of other regulatory proteins. The other overlapping open reading frame in X encodes two proteins with molecular weights of 27 and 21KDa. The 27 KDa protein known as *rex* is a regulatory protein which functions in the transport of HTLV structural mRNAs from the nucleus to the cytoplasm (Hidaka et al. 1988). The function of the smaller of the two proteins species is unknown.

Schematic Representation of HTLV-I Infection



SEROEPIDEMIOLOGY OF ATL

The etiologic association of HTLV-I with ATL was based partly on seroepidemiologic evidence from Japan where over one million people are infected with this virus and 500 cases of ATL are diagnosed yearly (Hinuma, 1986). However the development of ATL is rare occurring in less than one in 600 HTLV-I infected carriers (Tajima et al. 1986). As mentioned previously, the data revealing that ATL patients in southwestern Japan were seropositive for HTLV-1 were the first clues that such an association existed (Hinuma et al. 1981; Yoshida et al., 1982). More detailed seroepidemiologic testing of the Japanese population has indicated that there is a variable incidence for HTLV-I infection among cities within the endemic region ranging from 35% among Okinawans to as low as 0.3% to 1.2% in nonendemic areas (Hinuma, 1986). This clustering effect is thought to be due to the limited transmission of HTLV-I between socially isolated population centers. The origin of HTLV-I in Japan is not known. Some investigators have hypothesized it may have been brought to Japan by the original Joman settlers, who arrived in Japan between 300 and 10,000 BC and settled in areas of Japan where HTLV-I is endemic (Ishida and Hinuna, 1986). Some investigators have postulated an African origin for HTLV-I, suggesting that the virus may have been brought to Japan from Africa by traders. The prevalence of HTLV-I and the very closely related simian T cell leukemia virus in Old World monkeys, such as Japanese macaques, suggest that the virus infected both man and Old World primates from a common source (Homma et al. 1984).

Detailed seroepidemiologic testing of clinically normal Japanese individuals has shown that the pattern of HTLV-I transmission is through one of three different modes. The virus can be transmitted from male to female during sexual intercourse via HTLV-I infected cells in semen. Also, mothers infected with HTLV-I can transmit the virus to the fetus or newborn and this is likely a major source of viral transmission (Robert-Guroff et al. 1983). The mode of maternal-fetal transmission is controversial, but is thought to occur through passage of infected lymphocytes transplacentally or in breast milk (Komuro et al. 1983). In one study comparing bottle-fed infants to breast-fed infants of HTLV-I infected mothers, 11 of 24 breast-fed infants acquired the infection, as opposed to only 1 of 11 of bottle-fed babies (Ando et al. 1987). Hence prenatal screening might limit the transmission of the virus. The last major route of transmission is through blood products involving the passage of cells from donor to recipient (Okochi et

al. 1984). Based on these data, it appears that HTLV-I transmission requires the presence of infected cells in semen, breast milk, or blood products, as opposed to the human immunodeficiency virus which can be transmitted in cell-free fluids.

The prevalence of both HTLV-I and HTLV-II infection appears to be increasing in Western Europe and the United States, particularly among intravenous drug abusers, and possibly, homosexuals. A recent serologic study of a cohort of intravenous (IV) drug abusers in New York reported a prevalence of 9% for HTLV-I, 18% for HTLV-II, and 41% for HIV (Robert-Guroff et al., 1986). In a recent study in Trinidad, an endemic locale for HTLV-I, 15% of homosexuals were seropositive for HTLV-I as opposed to 2.4% of the general population (Bartholomew et al., 1987). Interestingly although HIV was only recently introduced into Trinidad, approximately 40% of homosexuals were found to be infected with this virus, compared with <1% of the general population. These studies suggest that in Western countries, HTLV-I, and HTLV-II, are spreading in some of the same population groups at risk for infection with HIV. However, HTLV-I and HTLV-II appear to spread less efficiently than HIV, as noted by the relatively rapid rise in rates of HIV seropositivity as compared to HTLV.

HTLV-I TRANSMISSION BY BLOOD PRODUCTS

Because studies have shown that healthy adults with anti-HTLV-I antibodies are carriers of HTLV-I and that the virus can be cultured from their peripheral blood lymphocytes, numerous studies have sought to examine the role of this virus in blood transfusion. In 1984, Okochi and associates showed that of 41 recipients of whole blood or blood components that contained cells from donors with antibodies to HTLV-I 26 (63%) had seroconversion. No seroconversion occured after administration of fresh-frozen plasma prepared from donors positive for anti-HTLV-I. None of 252 recipients of antibody-negative blood had seroconversion. Antibodies became detectable 3 to 6 weeks after transfusion with anti-HTLV-I positive units. IgM antibodies were detected early after transfusion but became undetectable within a few months. In several months, IgG antibody determinations became equivalent to those in patients with ATL and persisted without diminution. It is estimated that 50,000 Japanese have been infected with HTLV-I through blood transfusions (Seto and Okochi, 1986).

HTLV-I can be transmitted by cellular blood products including whole blood, erythrocytes, platelets, and leukocyte preparations. Morishima and

associates found that in 97% of HTLV-I antibody-positive donors, the virus could be demonstrated in cultured lymphocytes from concentrated erythrocyte products, whereas none of the antibody-negative units was culture positive (Morishima et al., 1986). Blood derivatives such as coagulation factor concentrates seem free of transmission risk relative to cellular blood products, although their safety cannot be absolutely ensured. A study of HTLV-I antibody status in 85 Austrian patients with hemophilia treated with factor concentrates prepared in the United States, 24 patients with hemophilia from Georgia treated with concentrates prepared in Georgia, and 10 patients with hemophilia and AIDS in the United States showed only 1 antibody-positive patient among the 119 studied (Chorba et al., 1985). This finding may indicate that HTLV-I is not readily transmitted by lyophilized factor concentrates.

Sato and Okochi isolated human T cell clones bearing antigens encoded by HTLV-I from six patients who produced antibodies against HTLV-I after receiving HTLV-I positive cellular blood components. The presence of viral antigen was demonstrated by reactivity with HTLV-I monoclonal antibody to the gag antigens p19 and p24 (Sato and Okachi, 1986). The clonal cell lines had the same surface markers as neoplastic cells of ATL and had the HLA phenotype of the recipients themselves. Proviral DNA of HTLV-I was demonstrated in each of the clonal cell lines. Kotani and colleagues were able to transmit HTLV-I for five passages from rabbit to rabbit by transfusion of whole blood or washed blood suspensions but not by infusion of cell-free plasma. Seroconversion occurred in the rabbits 2 to 4 weeks after blood transfusion (Kotani et al., 1986).

Transmission of HTLV-I by transfusion of blood products can be summarized as follows: cellular blood products from seropositive donors are infectious, HTLV-I can be transmitted by blood transfusion, and recipients in whom seroconversion has occurred are in a state of persistent infection. Currently, ELISA tests for HTLV-I and HTLV-II antigens have become part of routine blood bank screening to prevent widespread dissemination of these viruses into the general population.

CLINICAL SYNDROMES OF HTLV-I INFECTION

Adult T cell leukemia (ATL)

The host response to infection with HTLV-I can be manifest in a number of ways. The infected individuals can be asymptomatic, develop various forms of T-cell leukemia, develop a degenerative neurological syndrome known as tropical spastic paraparesis, or develop arthropathy. It is not clear whether host or viral factors are the critical factors in determining the outcome of HTLV illness. The stages of ATL are termed (a) a symptonetic carrier state (pre-ATL) (b) preleukemia state (pre-ATL) (c) chronic smoldering ATL, and (d) acute ATL (Kinoshita et al., 1985; Shimoyama et al., 1991). The majority (98 to 99 percent) of infected persons will have no signs or symptoms of disease (Hinuma et al., 1981 and 1982; Blattner et al., 1983). There are some data to suggest that the chance of ATL developing after seroconversion is 0.1 to 1.0 percent (Kondo et al, 1987). The carrier state, however, should not be considered innocuous, as these patients may remain infectious and may have progression to more obvious disease states. The stages of ATL are termed (a) asymptomatic carrier state (b) preleukemic state (pre-ATL) (c) chronic or smoldering ATL and (d) acute ATL (Kiyoshita et al., 1985; Shimoyamo et al., 1991). However ATL does not usually occur until 20-30 years after infection (Kawano et al., 1985). In the asymptomatic carrier state there may be an increase in serum immunoglobulin and although leukocyte counts and differentials are normal, the percentage of CD4 and Tac positive cells may be elevated (Yasuda et al., 1986).

Patients with subclinical or pre-ATL are almost entirely asymptomatic and are distinguished by a mild proliferation of abnormal-appearing lymphocytes (Kinoshita et al., 1985; Kawano et al., 1985). The leukocyte count is normal, and transient fever or rash may occur. Minor lymphadenopathy may be present; lymph node biopsy specimens are typically unrevealing. The bone marrow is infrequently involved and the patients feel well. Evidence of HTLV-I infection, either by seropositivity or proviral incorporation, is present. In roughly 50 percent of these patients, the lymphocytosis will resolve apparently without residua; the remainder will have persistence of this pattern or will show progression to a more acute form of the disease.

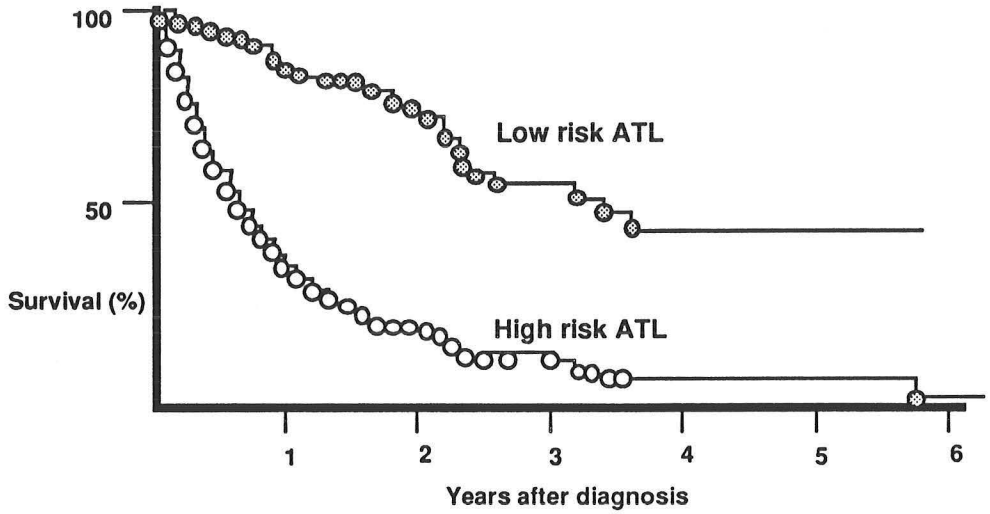
Clinical Phases of HTLV-I Infection

	Monoclonal virus	Visceropathy	Skin lesions	WBC	Course
Carrier	+	-	-	**Normal**	-
Pre-ATL	+	-	-	**10,000**	**Variable**
Smoldering ATL	+	+	-	**Normal**	**Variable (→ATL)**
Chronic ATL	+	+	-	**>10,000**	**Variable (→ATL)**
Acute ATL	+	+	+	**100,000**	**6-8 months**

The chronic or smoldering form of ATL is associated with cutaneous lesions (erythema, papules, and nodules), antibody to HTLV-I, mild abnormal lymphocytosis, and prolonged survival (Yamaguchi et al., 1983; Kinoshita et al., 1985; Kawano et al., 1985). Nearly a third of patients with clinically apparent HTLV-I infection will fit this description. Bone marrow involvement is slight; hypergammaglobulinemia is common. Of note, hypercalcemia, lymphadenopathy, and hepatosplenomegaly are absent. Chromosomal markers can be useful in determining the progression of this chronic or smoldering form of disease to ATL (Fukuhara et al., 1983). Those patients with a normal karyotype have long survivals. Progression to ATL is frequently associated with trisomy of chromosome 3 and 7 or loss of the X chromosome. Several studies indicate the possibility of immunosuppression during this stage of the disease which can lead to morbidity and mortality. Forty percent of patients with smoldering ATL progress to acute ATL.

The development of acute ATL is a common clinical manifestation of HTLV-I infection (Uchiyama et al., 1987; Blayney et al., 1983; Bunn et al., 1983). Unexplained rashes, cough and malaise, adenopathy, and abdominal complaints, weakness, rash, and fever are presenting complaints. In general, the clinical picture is characterized by skin lesions, generalized lymphadenopathy, hepatosplenomegaly, lymphomatous meningitis, an elevated leukocyte count with multilobed lymphocytes, and hypercalcemia. Presenting complaints of patients suffering from ATL include abdominal distension/pain in 43 percent,

Survival Curves of Patients



cutaneous lesions in 39 percent, fever in 39 percent, lassitude in 36 percent, cough in 18 percent, and lymphadenopathy in 11 percent (Matsumoto et al., 1979). A summary of the clinical and laboratory characteristics of patients presenting with ATL is shown below (Kim and Durack, 1988). Hematologically, the patients with acute ATL present with leukocytosis, demonstrating a predominance of lymphocytes with pleomorphic nuclei. Anemia and thrombocytopenia are infrequent and typically mild but eosinophilia may be prominent. The acute form of ATL is rapidly fatal with a median survival rate of eight months (Bunn et al. 1983; Catovsky et al., 1982).

The cutaneous lesions associated with ATL are similar in appearence to those found in the cutaneous T-cell lymphomas (CTCL), (mycosis fungoides and the Sezary syndrome), hence the early confusion of the two entities (Poiesz et al., 1980 and 1981). Generalized erythroderma, flesh-to-violaceous-colored papules, nodules, plaques, and maculopapular rashes have been described (Broder et al., 1984; Uchiyama et al., 1987;). Differentiation between ATL and CTCL can usually be made histologically. ATL should be considered in cases of rapidly progressive CTCL.

Other clinical manifestations include pulmonary complications which were noted in one series among 26 of 29 patients with ATL (Yoshioka et al., 1985). Clinical and roentgenographic evidence of progressive pulmonary impairment are common; leukemic infiltrates account for 50 percent of these cases, infectious etiologies the remainder. Previous studies emphasize the susceptability of these

Clinical and Laboratory Characteristics of Patients with ATLL

Manifestation	%
Lymphadenopathy	93
Hepatomegaly	75
Splenomegaly	63
Skin lesions	56
Bone marrow	75
Opportunistic infection	80
Leukocytosis	100
Hypercalcemia	90
Abnormal liver function test results	31
Elevated lactic dehydrogenase	100
Lytic bone lesions	50

patients to infection, both intrinsically and as a result of cytotoxic treatment regiments (Matsumoto et al., 1979; Bunn et al., 1983). Opportunistic pathogens such as Pneumocystis, cytomegalovirus, and Candida are frequently found at necropsy, as is infection with the usual bacterial pathogens such as Pseudomonas. Hepatomegaly with jaundice was found in 23 percent of the patients in one series due to infiltration of the liver by tumor cells (Jaffe et al., 1984). Roughly one fourth of the patients from the National Cancer Institute had leptomeningeal involvement with altered mental status; cerebrospinal fluid glucose and protein values were normal, and the diagnosis was made after repeated cytologic examination of the spinal fluid (Bunnet et al., 1983).

Lytic lesions of the skull and long bones are common (17 to 50 percent), though radionuclide scanning suggests a diffuse increase in skeletal bony turnover (Broder et al., 1984). The hypercalcemia associated with ATL has been attributed to increased osteoclast and osteoblast activity. Studies of parathyroid hormone, vitamin D metabolites, prostaglandins, and osteoclast activating factor have been unrevealing or equivocal (Grossman et al., 1981). Recently it was demonstrated that the HTLV-I regulatory protein, *tax*, is capable of activating

gene expression from the osteoclast activating factor gene. This suggests that viral proteins can activate cellular genes which regulate calcium metabolism.

Histopathology of ATL

An important feature of ATL is the presence of markedly pleomorphic lymphoid cells in the peripheral blood (Uchiyama et al., 1987; Catovsky et al., 1982; Bunn et al., 1983). The transformed lymphocytes from any single patient are derived from a dominant clone of malignant cells as evidenced by the presence of a single rearrangement of the T-cell receptor β gene (Jarrett et al., 1986) and the provirus in the cells is integrated in an oligoclonal fashion (Yoshida et al. 1982 and 1984; Hoshino et al., 1983). These cells have moderately condensed nucleoli chromatin, inconspicuous nucleoli, and a markedly irregular nuclear contour in which the nucleus is divided in several lobes. Cells with such markedly pleomorphic nuclei are very characteristic of HTLV-associated disease and can be readily distinguished from Sezary cells and cells of other mature and immature T-cell malignancies. However, in approximately 20%, the nuclear irregularities are less extreme and a distinction from Sezary syndrome on morphologic grounds alone is difficult (Broder et al., 1984).

Characterization of Adult T-cell Leukemia Cells

1) **All cells anti-T4 reactive**

2) **Variability of T3,T11, and T12 antigens**

3) **Inducer of T8 precursors of suppressor cells**

4) **Deficient in NK activity**

5) **Chromosomal abnormalities include trisomy 3 and 7 in addition to $6q^-$ and $14q^+$**

The histopathologic features seen in lymph nodes and other tissues of patients suffering from ATL are even more varied than the cytologic findings in the peripheral blood cells. ATL may be associated with lymphomas of several histologic subtypes. The variety of histopathologic features is not indicative of a difference in cellular origin or disease process. Moreover the histopathologic features of virus-positive leukemia/lymphoma, cannot always be distinguished from those of virus-negative cases on morphologic grounds (Broder et al., 1984). The classification of 32 virus-negative cases of peripheral T-cell lymphoma did not show significant differences from the 13 virus-positive patients described above (Broder et al., 1984). However, the virus-negative lymphomas more frequently contained an inflammatory background of eosinophils, plasma cells, epithelioid histiocytes, or all, and lacked a leukemic pattern of infiltration. Thus, the most specific morphologic feature of ATL appears to be the presence of polylobed and pleomorphic lymphoid cells in the peripheral blood of patients with virus-positive malignancies.

HTLV-I and Mycosis Fungoides

1) **Mycosis fungoides is an indolent cutaneous lymphoma comprised of $CD4^+$ and Tac^- cells**

2) **Presence of deleted forms of HTLV-I in some cases**

3) **Patients frequently seronegative for HTLV-I**

A controversial aspect of the classification of retrovirus-associated lymphoid malignancies has been their relation to mycosis fungoides/Sezary syndrome. Cutaneous involvement is frequent in ATL being present in 66% of patients in the Hanaoka series and 64% of those in the National Cancer Institute series (Jaffe et al., 1984). Furthermore, focal epidermal infiltration or Pautrier's microabscesses were seen in two thirds of patients with cutaneous involvement

(Hanaoka et al., 1979). The acute course and absence of a chronic premycotic phase in almost all patients with ATL helps in differentiating it from patients with typical mycosis fungoides. The distinction between ATL and Sezary's syndrome has become less clear recently. A number of cases of Sezary syndrome have been identified which contain defective proviral forms of HTLV-I integrated into the tumor cells. These defective proviruses may delete portions of the gag or env genes and the patients are frequently HTLV-I negative by ELISA (Hall et al., 1991). The significance of these defective provirus in the pathogenesis needs to be considered. Furthermore 18 of 20 cases of classical mycosis fungoides were found to contain retroviral particles similar to HTLV by electron micorscopy of tumor specimens (Zucker-Franklin et al., 1991). Though mycosis fungoides and ATL are unique clinical entities, it is possible that many cases of mycosis fungoides are due to retroviral infection by HTLV or related viruses.

Other pathologically proven sites of involvement of ATL include bone marrow, liver, lung, and cerebrospinal fluid (Broder et al., 1984; Shimoyama et al., 1991). Patients may have peripheral blood involvement in the absence of bone marrow involvement. In those patients with positive findings from bone marrow biopsies, the degree of marrow replacement is less than expected, given the high levels of circulating abnormal cells. When present, marrow involvement is usually not paratrabecular, as seen in the follicular lymphomas. Biopsy specimens of lytic bone lesions usually fail to show involvement by tumor and show only microcystic resorption and increased osteoclastic activity. Increased osteoclastic activity is also seen in routine iliac crest biopsy specimens supporting the concept that a humoral factor is responsible for the osteoclast activation, bone resorption, and hypercalcemia.

Treatment of ATL

No treatment is warranted for HTLV-I positive patients who are asymptomatic or who have subclinical or chronic forms of ATL (Kawano et al., 1985; Kuefler and Bunn, 1986;). Chemotherapy though not resulting in long term survival is the standard treatment for acute ATL. Treatment with aggressive multiagent non-Hodgkins lymphoma protocols such as Pro-MACE-MOPP met with some success (Bunn et al., 1983). Bunn treated 10 patients with such a regime and 7 achieved a complete remission. However five of these patients relapsed within a year with the emergence of a more immature T-cell phenotype

(Bunn et al., 1983; Yamada et al., 1984). Treatment with the adenosine deaminase inhibitor, 2-deoxycoformycin, has been reported to induce remission in selected patients (Daenen et al., 1984).

Monoclonal antibodies to the interleukin-2 receptor (anti-Tac) have been used to treat HTLV-I infected patients because of the high numbers of these receptors present on HTLV-I transformed T cells. Several patients treated by Waldmann achieved short term remissions using this antibody (Waldmann et al., 1985). To enhance the cytotoxicity of these antibodies they have been tagged with a variety of substances including ricin, pseudomonas exotoxin, and radioactive bismuth (Fitzgerald et al., 1984; Kronke et al., 1985 and 1986; Kozack et al., 1986). Though these antibodies have been effective in reducing the proliferative capacity of HTLV-I infected cells in vitro, it is not clear whether these antibodies will have efficacy in patients with ATL.

Therapy of ATL

1) **Combination chemotherapy**

2) **2-deoxycoformycin**

3) **Azidothymidine**

4) **Anti-IL-2 receptor antibody (Anti-Tac)**

5) **Vaccines**

No effective vaccines against HTLV-I have been developed. However immunization of monkeys with env proteins produced by bacterial expression vectors is able to protect monkeys against primary HTLV-I infection (Fischinger et al., 1985; Nakamura et al., 1987;). Given the relative infrequency of HTLV-I leukemic transformation and the long latency period involved in the development of ATL, the assessment of vaccine efficiency will be difficult. Thus it is likely that prevention of ATL will focus on the development of antiviral drugs rather than vaccines.

Tropical Spastic Paraparesis

Tropical spastic paraparesis (TSP) or HTLV-I associated myelopathy (HAM) is a slowly progressive encephalomyelopathy that generally presents in the middle adult years (Gessain et al., 1985; Osame et al., 1986; Osame et al., 1987; Vernant et al., 1987). The initial symptoms are increasing difficulty in walking, low back pain, and cramping sensations of the legs. Urinary sphincter dysfunction is also common. Impotence or diminished libido is almost universal. Most cases reach a clinical plateau within two years of the start of the symptoms and remain at that level of function without remission for years.

Symptoms at Onset of TSP

Features	%
Leg weakness, heaviness	100
Parasthesias in legs, feet	98
Bladder, bowel problems	96
Penile impotence	93
Pain, cramps, legs and back	85
Eye complaints, including "blurred" vision, transient	37
Fever, "flu"	10
Parasthesias in arm	5
Type of onset	
Gradual	87
Acute	13
Form of onset	
Asymmetrical	64
Symmetrical	36

Clinical analysis of patients with TSP reveal that the syndrome is dominated by spastic paraparesis with signs of pyramidal tract involvement affecting the legs (Roman and Roman, 1988). Spasticity is moderate and

predominates on the thigh adductors, and to a lesser degree on the thigh extensors and gastrocnemius muscles. The combination of pyramidal proximal motor weakness of the lower limbs and spasticity results in a typically slow scissoring gait, with a deliberate dragging and shuffling of the feet. The knee reflexes are pathologically increased with the presence of crossed adductor responses and ankle clonus. Signs of peripheral neuropathy including stocking type loss of sensation occur in 20-50% of patients. However, considering the extent of the pyramidal lesion, the overall involvement of the peripheral sensory and motor neuropathy are comparatively minor. The most common differential diagnostic dilema of a chronic spastic myelopathy in middle age patients is multiple sclerosis. Other less likely possibilities include subacute combined degeneration from vitamin B12 deficiency, syphlis, parasitic, and toxic etiologies. Treatment with corticosteriods frequently ameliorates the symptoms of TSP and helps prevent progression of the disease (Dixon et al., 1990).

The basic neuropathological lesion in TSP is a chronic inflamatory process involving mainly the white matter of the spinal cord and less commonly the brain. The primary changes are astrocytic gliosis, capillary proliferation, perivascular lymphocyte cuffing, and loss of myelin and axons predominantly in the pyramidal, spinocerebellar and dorsal columns of the spinal cord. Similar but less striking changes are found in the medulla, pons, and in the white matter of the cerebellum and cerebrum of these patients (Akizuki et al., 1987).

Groups of patients with this disorder were initially recognized in southern India, Jamaica, South Africa, and Colombia (Gessain et al., 1985; Osame et al., 1986; Vernant et al. 1987; Roman and Roman, 1988;). The geographic clustering and sporadic appearance of the neurologic syndrome led to the finding of antibodies to HTLV-I in the blood of patients with TSP/HAM (Bhagavati et al., 1988). Seropositivity to HTLV-I was subsequently confirmed in a number of Jamaican and Colombian patients with the syndrome, and in addition antibodies to HTLV-I were found in their cerebrospinal fluid (Gessain et al. 1985). Soon afterward patients living in nontropical areas of southwestern Japan, which were endemic for HTLV-I were also found to have clinical symptoms of TSP/HAM. Subsequently, cases of TSP/HAM associated with HTLV-I have been reported world-wide including a number of cases in the United States.

Persuasive evidence for a closer association of disease and virus came with the isolation of HTLV-I from cerebrospinal fluid lymphocytes of a patient in Japan with TSP/HAM who had both serum and cerebrospinal fluid HTLV-I antibodies

(Hirose et al., 1986; Libersti et al., 1988). Electron microscopy demonstrated HTLV-I like viral particles in pathologic spinal cord tissue from a Jamaican patient with TSP/HAM. Intact HTLV-I virus has been produced using peripheral lymphocytes from healthy controls cocultured with irradiated T lymphocyte cells derived from the cerebrospinal fluid of patients with TSP/HAM (Jacobson et al., 1988). Both Southern blot hybridization and electron microscopy of cultured T lymphocytes isolated from cerebrospinal fluid of patients with TSP/HAM has revealed HTLV-I in a proviral form in these cells (Yoshida et al., 1987). Intrathecal synthesis of HTLV-I antibodies and oligoclonal bands in cerebrospinal fluid specimens of TSP/HAM patients has also been demonstrated (Gout et al., 1989). However, to date, only one patient has been reported who had both ATL and TSP: he was a Trinidadian man who had TSP/HAM for 16 years before ATL developed (Bartholomew et al., 1986).

Arthropathy

Chronic arthropathy has been associated in some patients with HTLV-I infection (Sato et al. 1991). All of the patients were women who developed articular symptoms which were predominantly a chronic persistent oligoarthritis, with preferential involvement of the large joints. Arthroscopic examination revealed a focal and superficial distribution of the proliferating synovial villi, with minimal changes in articular cartilage. Roentgenograms showed minimal erosions in the affected large joints. The most striking feature of this arthritis was the preferential accumulation of ATL-like cells in both the synovial fluid and tissue, and the presence of HTLV-I proviral DNA in synovial fluid and tissue cells.

In these patients high titers of antibody against HTLV-I were detected in both sera and synovial fluids (Sato et al., 1991). Atypical lymphocytes consistent with ATL-like cells were predominant in synovial fluids and/or synovial tissues with HTLV-I proviral DNA integrated into these cells. Most patients had a history of blood transfusion prior to the onset or acceleration of the arthopathy. The geographic distribution of the patients is in areas endemic for ATL and HTLV-I. Given the above features, the arthropathy appears distinct from other rheumatic diseases although the possibility of an incidental complication of rheumatic disorders in HTLV-I carriers cannot be excluded Myalgia and frank myositis has also been associated with HTLV-I infection (Wiley et al., 1989; Sato et al., 1991).

What is the pathogenesis of this arthropathy? HTLV-I infected T cells may directly stimulate the proliferation of synovial cells leading to arthropathy. Phenotypic analysis of lymphocytes from synovial tissue reveals a predominance of activated CD8-positive T cells suggesting that immune cell interactions with HTLV-I infected cells may play an important role in the arthropathy. Another possible pathogenetic mechanism is that the cytokines produced as a result of the HTLV-I infection may cause the arthropathy. Salahuddin et al. reported that HTLV-I transformed cells produce multiple lymphokines that either directly or indirectly activate the synovial cell proliferation seen in the arthropathy (Salahuddin et al., 1984). Finally, HTLV-I infection may modify the underlying arthropathy by having HTLV-I infected cells accumulate at the inflammatory site as a secondary event and modifying the immune response in the involved joint cavity.

CLINICAL MANIFESTATIONS OF HTLV-II

HTLV-II was initially identified in a patient suffering from a T-cell variant hairy cell leukemia (Kalyanaraman et al., 1982). Another patient with a similar clinical presentation was also found to harbor HTLV-II (Rosenblatt et al., 1988). Other patients with T-cell CLL and T-cell prolymphocytic leukemia were also found to be infected with HTLV-II.. The clinical course of these patients varied from asymptomatic to rapidly progressive. The presence of integrated HTLV-II proviral DNA in tumor cells further suggests HTLV-II as an etiologic agent of T-cell neoplasia. Therefore an etiologic relationship between HLTV-II and T-cell malignancy has been suggested.

The largest study of HTLV-II infection was performed on a group of 21 intravenous drug users (Rosenblatt et al., 1990). High levels of HTLV-II provirus were detected in purified T cells from those patients using PCR analysis, with little or no HTLV-II proviral DNA found in B lymphocytes or monocytes. The frequency of HTLV-II-infected cells was approximately 1 in 500 cells which is a much higher proportion of cells infected than with HTLV-I. Most of these HTLV-II infected individuals were asymptomatic though several manifested a benign elevation of their T lymphocyte count. The long term consequences of HTLV-II infection in this patient population remains to be seen.

HTLV-II and Clinical Disease

1) **Rare T-cell malignancies including hairy cell leukemia**
2) **Relatively high prevalence among drug abusers**
3) **Number of infected cells 1/100 to 1/500**
4) **Most individuals asymptomatic though benign lymphocytosis occurs**

HTLV DIAGNOSIS

Knowledge of HTLV seropositivity may help to prevent transmission between sexual partners, mother to child, and by blood transfusion. Furthermore it is critical in the diagnosis of HTLV-I induced leukemia and myelopathy. The enzyme linked immunosorbant assay, ELISA, is currently frequently used for screening purposes. This ELISA is directed against the gag proteins p19 and p24. The HTLV-I ELISA will identify both HTLV-I and HTLV-II infected individuals. Cross reactivity with HIV infected individuals is not a problem, due to the lack of homology between HTLV and HIV. Sera that test positively for HTLV antibodies by ELISA should be confirmed by direct radioimmuno-precipitation assays or Western blots (Rosenblatt et al. 1988).

A variety of viral proteins in HTLV-I and HTLV-II are immunogenic, and elicit an antibody response in patients. These include structural proteins encoded by the *gag* genes as well as viral regulatory gene products including *tax*. Occasionally, patients have limited or absent reactivity with gag and env antigens, but react strongly against the nonstructural protein, *tax*, allowing detection of seropositive individuals in whom the ELISA assay is negative (Ehrlich et al., 1989). It is possible useful prognostic information can be determined from the pattern of seroreactivity with different HTLV-I related antigens.

Finally, DNA amplification techniques have increased the sensitivity of molecular diagnostic methods. The polymerase chain reaction (PCR) requires

only a small number of HTLV-infected cells, because it specifically amplifies the proviral signal by a factor of greater than 10^5 to levels that can not be detected by Southern blotting. This method has been used to identify the mutations in a variety of genetic diseases including sickle cell anemia and thalassemia, as well as the presence of HIV-infected patients. PCR has recently been successfully used to identify the presence of HTLV-I or HTLV-II in patient derived samples (Bhagavati et al., 1988; Rosenblatt et al., 1991). It has both a high degree of sensitivity and specificity and allows the discrimination of HTLV-I and HTLV-II which is not seen in ELISA asays.

IN VITRO TRANSFORMATION

The ability of HTLV-I and HTLV-II to transform normal peripheral lymphocytes in vitro, as defined by immortalization of a population of lymphocytes with helper/inducer T cell phenotype, provides further evidence that these viruses are the etiologic agents for T-cell leukemia. Typically, transformation is accomplished in a co-cultivation system in which lethally irradiated HTLV-infected cells are incubated with normal human leukocytes (Miyoshi et al., 1981; Chen et al., 1983). Infection and transformation of normal cells using cell-free supernatants containing HTLV-I and HTLV-II are not efficient. The transformed lymphocyte populations emerge within 2 to 4 weeks of co-cultivation and continue to grow in the absence of interleukin-2 (IL-2).

Originally, only T cells were thought to be infected by HTLV, but studies indicate that a variety of lymphoid and nonlymphoid cell lines, such as Epstein-Barr virus (EBV)-transformed B cell lines (729-6, Raji), HL-60 cells, HeLa cells, HOS (osteosarcoma) cells, Vero (epithelioid) and human endothelial cells, can be infected (Wachsman et al., 1986). In addition, the host range for HTLV infection extends beyond human lymphocytes to that of rat, rabbit, cat , mouse, and Japanese macaque (Miyoshi et al., 1983). As yet, transformation has been observed only in the T cells of these species. Thus, although non-lymphoid cells have the biochemical apparatus for HTLV replication, transformation is confined to the T lymphocytes.

Even though the immunologic phenotype of leukemic cells in ATL is similar to the in vitro transformed lymphocyte population, the relationship between in vitro transformation and in vivo leukemogenesis is unknown. Some aspects of the biology of cells obtained from in vivo versus in vitro transformation

are different. For example, in contrast to in vitro transformed T cell lines that normally express genomic and subgenomic viral RNA, fresh ATL tumor cells do not express detectable levels of viral RNA until cultured in vitro (Rosenblatt et al., 1988; Kim and Durack, 1988). Also, based on seroepidemiologic data, only a small percentage of the HTLV-I infected population will develop leukemia, and the latent period for leukemogenesis appears to be two to three decades while in vitro transformation is rapid and occurs with nearly all HTLV-I isolates (Hinuma et al. 1981; Kuefler et al., 1986; Rosenblatt et al., 1988). These observations suggest that in addition to HTLV infection, other events or cofactors are likely to be required for leukemic transformation in vivo.

What mechanisms function to induce ATL? Current evidence suggests that the HTLV-I regulatory protein *tax* plays a key role. *Tax* is a potent activator of viral transcription and also activates the expression of certain cellular genes including IL-2, the IL-2 receptor, and the *c-fos* proto-oncogene (Cann et al., 1985; Greene et al, 1986; Siekevitz et al. 1987). The central role of these genes in normal T-cell activation and growth suggests that *tax* activation of these cellular transcription units may represent an important mechanism by which HTLV-I initiates T-cell transformation.

HTLV-I Tax Protein

1) **40 kDa nuclear protein**

2) **Activates HTLV-I gene expression**

3) **Activates cellular gene expression of IL-2 and IL-2R**

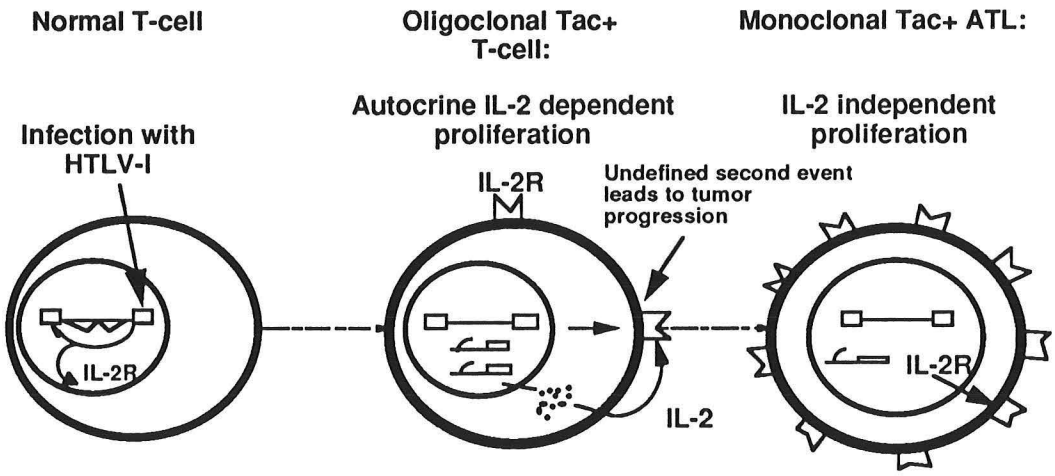
4) **Represses gene expression of some cellular genes**

5) **Transforms T-lymphocytes and cooperates with ras in transformation**

Several lines of evidence suggest that the *tax* protein has the ability to initiate cellular transformation. Specifically, introduction of the *tax* into primary T cells via an unrelated viral vector results in the constitutive high level expression

of functional IL-2 receptors (Grassman et al. 1989). In contrast to normal activated T cells, these *tax*-expressing T lymphocytes can be maintained in continuous long-term culture in the presence of exogenous IL-2 suggesting that *tax* may deregulate the normal transient nature of the T-cell growth response. The ability of *tax* to function in the transformation of nonlymphoid cells has also been demonstrated in other experimental systems (Tanaka et al., 1990).

T-cell Transformation



An attractive model for ATL includes an early period of *tax*-induced polyclonal T-cell proliferation, mediated by the deregulated expression of IL-2 and its receptor. This polyclonal T-cell proliferation may facilitate the occurrence of additional events which lead to the monoclonal outgrowth of an IL-2 independent population of leukemic T cells. Recent studies have demonstrated that *tax* represses the expression of B-polymerase, an enzyme involved in DNA repair (Jeang et al., 1990). Notably, ATL cells are characterized by chromosomal breaks and other karyotypic abnormalities (Fukuhara et al., 1983). Although it remains unknown whether these chromosomal rearrangements are primary or secondary events in the cellular transformation process, these results raise the possibility that *tax* may mediate certain additional cellular events required for T cell transformation. Notably, ATL is distinguished from leukemia induced by most other replication competent retroviruses by both the lack of chronic viremia and absence of detectable expression of viral genes. Therefore, while it seems likely that *tax* may play an important role in the development of ATL, neither *tax* nor

other viral gene products may be required for the maintenance of this transformed phenotype. Thus ATL provides a good model system in which to undertstand the factors governing the development of human maligancy.

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