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H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

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分担研究報告書

悪性リンパ腫に対する免疫化学療法の最適化による新たな標準的治療の確立に関する研究

研究分担者：大間知　謙　（東海大学医学部血液腫瘍科　助教）

研究要旨：未治療進行期、国際予後指数で低リスク群のCD20陽性びまん性大細胞型B細胞性リンパ腫を対象として、rituximabとCHOP療法の併用において、国際的な標準療法である8コースのCHOP療法の各コースにrituximab を計8回投与する方法（R-CHOP療法群）を対照に、CHOP療法開始からrituximabを週1回連続8回投与する方法（RW+CHOP療法群）の有用性をランダム化第II/III相試験により検証する試験を開始した。
本研究は2007年12月から症例登録が開始された。2009年2月現在まで、37例の登録がなされている。当方は研究事務局としてプロトコールの改訂、各施設からの問い合わせに対する対応、病理中央診断のための標本回収を行ってきた。また、試験参加施設として、これまでに2名の適格基準を満たした患者に対してインフォームド・コンセントを行い、試験へ登録した。

A. 研究目的

現在の未治療進行期のCD20陽性びまん性大細胞型B細胞性リンパ腫（Diffuse Large B-cell Lymphoma：DLBCL）に対する標準的治療は、3週ごとのCHOP療法の各コースの第1日目にrituximabを計8回投与するものとされている（R-CHOP療法）。一方、わが国では2003年9月にB細胞性リンパ腫に対して承認されたrituximabの用法用量は375mg/m^{2}を1週間隔で8回の投与である。これは治験で行われた単剤での8週連続投与の有効性と安全性のデータに基づいている。rituximabの薬物動態は個体間差が大きく、血中半減期が11～572時間と通常の抗がん剤と違って著しく長くかつバラツキが大きいのが特徴であるが、3週毎の投与法での薬物動態に関する明確なデータは存在しない。rituximabの薬物動態と有効性の関連については、奏効例はrituximabの血中濃度が高いこと、血中rituximab濃度が高い例の無増悪生存期間（PFS）が長いことが知られている。これらの報告はrituximabの血中濃度を高めに維持すること、化学療法と同時併用することが治療効果の向上をもたらす可能性があることを示唆している。進行期高悪性度非ホジキンリンパ腫は治療初期の10～12週で完全寛解（CR）が得られない場合、それ以降にCRが得られても長期のPFSを得られる可能性が低い。治療初期に集中的にrituximabを投与する治療法は、間欠的にrituximabを投与するよりも有効性が高いことが期待される。そこで、R-CHOP療法の治療効果向上を目指して、CHOP療法との併用におけるrituximabの至適投与方法を多施設共同のランダム化比較試験により検討する。

B. 研究方法

本研究は未治療進行期、国際予後指数（international prognostic index:IPI）で低リスク群のCD20陽性DLBCL患者を対象として、rituximabとCHOP療法の併用において、8コースのCHOP療法の各コースにrituximabを計8回投与する方法（R-CHOP療法群）を対照に、CHOP療法開始からrituximabを週1回連続8回投与する方法（RW+CHOP 療法群）の有用性をランダム化第II/III相試験により検証するものである。

Primary endpoint
第II相部分：%CR
第III相部分：PFS
Secondary endpoints：PFS、全生存期間（overall survival：OS）、有害事象発生割合、重篤な有害事象発生割合、重篤な有害事象発生割合
予定登録例数：
第II相部分：RW+CHOP療法群で68例
第III相部分：各群180例、計360例（第II相部分を含む）
登録期間：5年、追跡期間：3年、総研究期間：8年
適格基準：
(1) 組織学的にDLBCLと診断されている
(2) 腫瘍細胞のCD20抗原が陽性
(3) 臨床病期がbulky massを有するII，III，IV期のいずれか
(4) 末梢血液中腫瘍細胞数が10000/mm3以下
(5) 年齢が20歳以上、79歳以下
(6) Performance Status（PS）：ECOG規準で0-2
(7) IPIでlow riskまたはlow-intermediate riskのいずれか
(8) 中枢神経系浸潤がない
(9) 測定可能病変を有する
(10) 以前に化学療法・放射線治療・抗体療法のいずれも受けていない
(11) 適切な臓器機能が保たれている
(12) 試験参加について患者本人から文書による同意が得られている
治療法：
A群（A法）：rituximab（tri-weekly）+ standard CHOP療法 = R-CHOP療法
rituximab（tri-weekly）375mg/m2 DIV 3週毎
・CHOPもrituximabもコース毎に投与する。
B群（B法）：rituximab（weekly）+ standard CHOP療法 = RW+CHOP療法
rituximab（weekly）375mg/m2 DIV 週1回・連続8回
・CHOPはコース毎に投与する。
・rituximabは第1コースのday1（治療開始日）を起算日として、day 1,8,15,22,29,36,43,50の計8回投与する。

standard CHOP療法（A群、B群共通）
Cyclophosphamide 750mg/m2（div）day 1
Doxorubicin 50mg/m2（div）day 1
Vincristine 1.4mg/m2（max 2.0mg/body）（iv）day 1
Prednisolone 100mg/body（65才以上では40mg/m2）（po）day 1-5
21日間を1コースとして、以上を計8コース繰り返す。

〔倫理面への配慮〕
ヘルシンキ宣言（日本医師会：http://www.med.or.jp/wma/）および臨床研究に関する倫理指針（厚生労働省告示第255号：http://www.mhlw.go.jp/topics/2003/07/tp0730-2.html）に従って本研究を実施する。
東海大学医学部では、2008年2月16日にIRBの承認を得た。IRBで承認が得られた説明文書を用いた説明と同意に基づいて患者を登録し、試験を実施する。

C. 研究結果

JCOG0601は2004年3月27日にJCOG運営委員会プロトコールコンセプトが承認された。と研究事務局として、その後プロトコール作成を行い、改訂を繰り返した後の2006年4月19日に、JCOGプロトコール審査委員会一次審査に提出した。その後、「NHLの効果判定規準の標準化国際ワークショップレポート」が改訂されたことを踏まえて、これに準じたJCOG版判定規準の改訂作業を行い、これを反映したプロトコールを作成した。本研究のプロトコールは2007年10月18日にJCOGプロトコール審査委員会で承認され、同年12月より登録が開始された。試験開始後、2回のプロトコール改訂を行い、現在も試験継続中である。2009年2月現在、37例が登録されている。当施設は、試験参加施設としても08年度中に2名の適格条件を満たした患者に対してインフォームド・コンセントを行い、同意を得て試験登録して治療を行っている。

D. 考　察

DLBCLに対する標準治療はR-CHOP療法であることが複数のランダム化比較試験の結果によって確立した。R-CHOP 療法におけるrituximabの使用法であるが、これまで報告された海外の試験では、rituximabの投与はCHOP療法の各コースの第1日目または2日前に、計8コース行われている。一方わが国でのrituximabの保険適応上の用法用量は375mg/m2を1週間隔で8回の投与である。我が国で行われたrituximabの治験から、奏効例はrituximabの血中濃度が高いこと、血中rituximab濃度が高い例のPFSが長いことが報告された。これらの知見は、rituximabの血中濃度を高めに維持することが治療効果の向上をもたらす可能性があることを示唆している。本研究における試験治療であるRW+CHOP療法は、CHOP療法の初期にrituximabを集中的に併用投与することでrituximabの血中濃度を高めることを目的としている。本研究によって化学療法と併用する場合のrituximabのより有用な投与方法が確立されれば、DLBCLに対する初回治療の他にもCD20陽性の悪性リンパ腫の寛解導入療法、再発時の治療法への応用も期待できる。

E. 結　論

本研究によって、進行期低リスクDLBCLに対するリツキシマブとCHOP療法の併用療法においてリツキシマブの有効な投与方法が確立できれば、国際的にも高い医学的貢献が期待できる。本研究は科学的にも倫理的にも適切にデザインされた、大規模な多施設共同の臨床試験として開始された。本試験を完遂することで、我が国の血液領域における臨床試験の基盤がより一層整備されることが期待できる。また本研究では、悪性リンパ腫の治療効果の判定にPETを用いた初めての臨床試験であり、本研究を通してPETの標準化の一助になることが期待できる。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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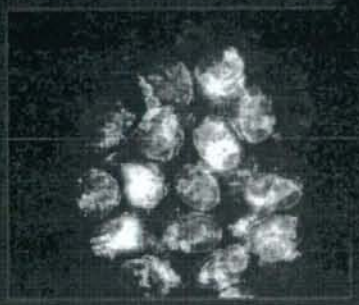

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14 Adult T-Cell Leukemia-Lymphoma

Kensei Tobinai and Toshiki Watanabe

SUMMARY OF KEY POINTS

Background
- Adult T-cell leukemia-lymphoma (ATLL) is a distinct peripheral T-cell malignancy that is associated with human T-cell leukemia virus type I (HTLV-I).

Virology
- HTLV-I is reverse-transcribed into DNA and integrated into the host cell.
- The HTLV-I genome encodes two unique regulatory proteins—Tax and Rex—that are responsible for viral expression and cellular transformation. Tax *trans*-activates viral and cellular genes that could be involved in the pathogenesis of ATLL.

Epidemiology
- The major cluster of HTLV-I-infected individuals and patients with ATLL exists in Japan, where approximately 1.2 million people are infected with the virus.
- Other clusters have been noted in the Caribbean islands (African), tropical Africa (African), South America (Mongoloid), and northern Oceania (Melanesian).
- HTLV-I is transmitted by mother to child through breast-feeding, by sexual contact, and by blood-borne transmission.
- The estimated cumulative risk of the development of ATLL in HTLV-I-positive individuals is 2.5%.

Clinical Manifestations
- Patients with ATLL show diverse clinical features, and four clinical subtypes have been recognized: acute, lymphoma, chronic, and smoldering types.
- The typical manifestations of acute-type ATLL include circulating neoplastic cells in the peripheral blood, generalized lymph node swelling, hepatosplenomegaly, skin involvement, and hypercalcemia.

Histopathology
- Leukemic cells in the peripheral blood characteristically show markedly polylobated nuclei, the so-called flower cells. Their immunophenotypes are CD4-positive and CD8-negative T-cell in most cases.
- All histopathologic specimens show the findings of peripheral T-cell lymphoma of various subtypes.

Diagnosis
- ATLL is suspected when the aforementioned characteristic clinical manifestations and/or the cytologic findings of leukemic cells in the peripheral blood are recognized.
- An immunophenotypic analysis of neoplastic cells and a serologic assay against HTLV-I are required for the clinical diagnosis of ATLL.
- The demonstration of the monoclonal integration of HTLV-I proviral DNA in the tumor cells can lead to a definite diagnosis of ATLL.

Treatment
- An accurate diagnosis of the clinical subtype is vital for appropriate decisions regarding treatment.
- Combination chemotherapies used in the treatment of non-Hodgkin's lymphoma are usually given to patients with the acute or lymphoma subtype of ATLL; however, most patients with ATLL are not curable with current chemotherapy regimens.
- Further efforts to incorporate new, innovative treatment modalities, such as new anticancer agents, monoclonal antibody therapy, molecular-targeting therapy, and allogeneic hematopoietic stem cell transplantation, are needed.

INTRODUCTION

Adult T-cell leukemia-lymphoma (ATLL) was first recognized in Japan in 1977s.[1] The disease was characterized as leukemia of peripheral T cells, generalized lymphadenopathy, hepatosplenomegaly, and skin involvement. Owing to its unusual geographic clustering in southwestern Japan, it was postulated that some infectious agent(s) had causative roles. Human T-lymphotropic virus (HTLV) was first isolated by Poiesz and associates[2] in the United States from cultured cells from one patient with an aggressive variant of mycosis fungoides and from one with Sézary syndrome. Although both patients were diagnosed clinically as having cutaneous T-cell lymphoma (CTCL) at the time of reporting, their clinical features were later found to closely resemble those of Japanese patients with ATLL.

In 1980, Miyoshi and coworkers[3] established the first cell line (MT-1) derived from neoplastic cells in an ATLL patient. They cocultured neoplastic cells from an ATLL patient with normal human cord blood lymphocytes and established the cell line MT-2 (derived from cord blood lymphocytes), which produced high amounts of type C retrovirus.[4] Using the MT-1 cell line, Hinuma and colleagues[5] found that patients with ATLL had antibodies against the virus-associated antigen in their sera. The "ATLL virus" was then isolated and characterized as an RNA retrovirus.[6] As HTLV and ATLL virus were found to be identical by a DNA sequence analysis, this virus was designated human T-cell leukemia virus type I or human T-lymphotropic virus type I (HTLV-I).[7]

The etiologic association of HTLV-I and ATLL is based on the findings that follow.

- The areas of high incidence of patients with ATLL closely correspond with those of high prevalence of HTLV-I carriers.[8]
- HTLV-I immortalizes T cells in vitro.[9]
- HTLV-I proviral DNA is detected in the neoplastic cells of ATLL.[10]
- Almost all patients with ATLL have antibodies against HTLV-I in their sera.

HTLV-I is the first retrovirus that was found to be associated with a malignant neoplasm in humans.

VIROLOGY AND PATHOGENESIS

HTLV-I is reverse-transcribed into DNA and integrated as a proviral DNA in the host cell. The HTLV-I provirus is 9.0 kilobases long and has structural genes in the order 5′-gag-pol-env-3′. Both ends of the HTLV-I proviral DNA contain repeats called long terminal repeats (LTRs). No specific integration sites of the HTLV-I provirus in the host cellular chromosomes have been identified.[11] A unique feature of the viral structure of HTLV-I provirus is the presence of a long sequence between *env* and 3′ LTR. One product of this *pX* gene, p40*tax*, acts on the LTRs for the *trans*-activation of the viral gene.[12]

The HTLV-I gene encodes three structural proteins: group antigen (*gag*), reverse transcriptase (*pol*), and envelope (*env*) proteins. The full-length mRNA is used for synthesis of *gag* and *pol* gene products. The gag protein is synthesized as a precursor polypeptide of 55 kilodaltons that is proteolytically cleaved into the individual gag proteins p19, p24, and p15. The protease is encoded in a different reading frame that spans the 3′ part of the *gag* region and the 5′ part of the *pol* region. The *pol* region encodes the reverse transcriptase, integrase, and RNase H. The *env* gene encodes two proteins made from a singly spliced mRNA. It is then cleaved intracellularly into an extracellular glycosylated protein (gp46) and a transmembrane (gp21). The *pX* region at the 3′ end of the genome has the potential to encode essential regulatory proteins (Tax and Rex) and three accessory proteins—p12, p13, and p30—that are important for viral infectivity and replication by influencing cellular signaling and gene expression.[13,14]

The life cycle of a retrovirus begins with the binding of the virus to specific receptors on the cell surface via viral envelope proteins. HTLV-1 is transmitted through a viral synapse and enters target cells via interaction with the glucose transporter GLUT1.[15] However, other molecules have also been reported to be involved in virus entry, for example, HSC70,[16] heparan sulfate proteoglycans,[17] and neurophilin-1.[18]

Role of Tax

The onset of ATLL is preceded by a long period of clinical latency, frequently lasting more than four decades. In addition, fewer than 5% of all infected individuals with HTLV-I develop ATLL. The promoter insertion model was rejected as the leukemogenic mechanism because integration sites of the provirus were random depending on the patient.[11] Consequently, a *trans*-acting viral factor, Tax, has been shown to be oncogenic, since it transforms and immortalizes rodent fibroblasts and T lymphocytes as well as human T lymphocytes. Tax *trans*-activates viral transcription through interaction with the cellular basic domain/leucine zipper transcription factors CREB and ATF-1. Tax interacts with numerous cellular proteins to reprogram cellular processes, including, but not limited to, transcription, cell cycle regulation, DNA repair, and apoptosis. Tax transcriptionally regulates cellular genes by interaction with enhancer-binding proteins such as CREB, NF-κB, and serum response factor and by tethering coactivators to the DNA-bound transcription factors. Tax also stimulates cell growth by direct binding to cyclin-dependent kinase holoenzymes and/or inactivating tumor suppressors such as p53 and DLG. Furthermore, Tax silences cellular checkpoints, which guard against DNA structural damage and chromosomal missegregation, thereby favoring the manifestation of a mutator phenotype in cells.[13,19]

Tax interacts and activates specific components of growth factor signal transduction pathways, such as IKK-IκB-NF-κB, RAS/mitogen-activated protein kinase, protein kinase A, and protein kinase C.[20,21] Interaction with IKKγ, a component of IKK complex, results in constitutive activation of this kinase complex.[21] Constitutive activation of the JAK-STAT pathway in HTLV-I-transformed cells has also been reported, although the mechanisms are not well understood.[23] Thus, HTLV-I infection results in aberrant activation of growth-promoting signaling pathways.

The oncogenic capacity of Tax has been reported in various systems; however, cellular transformation by HTLV-I in vivo is a multistage process, and viral gene expression is absent in ATLL cells in vivo.[24,25] Moreover, proviruses integrated in ATLL cells are frequently defective, have mutations in the coding region of Tax, and/or are methylated in the 5′ and 5′ LTR regions.[26–28] Thus, in addition to promoting growth directly, Tax should endow the infected T cells with capacities that aid the progression to transformed phenotypes in the absence of Tax. In this context, induction of a mutator phenotype by Tax in the infected cells appears to play an important role.[29] The roles of HTLV-I Tax in the multistep leukemogenesis of ATLL are illustrated in Figure 114-1.

Expression of antisense strand RNA with capacity encoding a zinc finger protein (HTLV-I basic leucine zipper factor) has opened a new research field. HTLV-I basic leucine zipper factor inhibits Tax-dependent viral transcription[30] and might be involved in growth of ATLL cells.[31]

Role of Chromosomal Abnormalities

Various karyotypic abnormalities have been reported in neoplastic cells of ATLL; however, no specific karyotypic abnormality has been

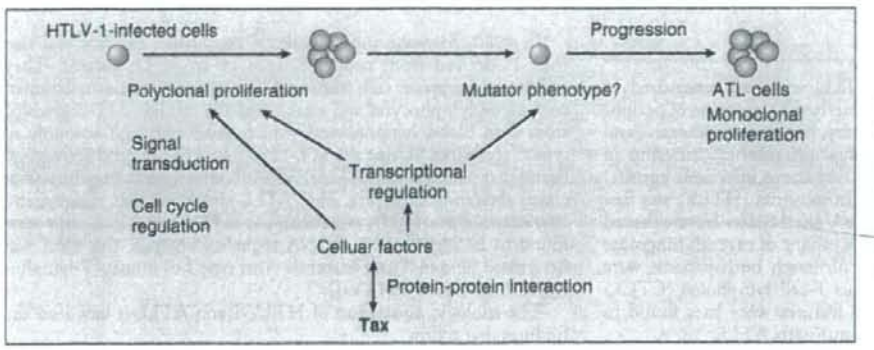

Figure 114-1 • Roles of HTLV-I Tax in the multistep leukemogenesis of ATLL. Tax exerts its biologic effects mainly through protein-protein interaction, resulting in deregulation of transcription, cell cycle control, and signal transduction. It also impairs the cell's ability to repair DNA damage, which can lead to the mutator phenotype of the infected cells.

and. In general, the chromosomal abnormalities are more complex the acute type compared with those in the chronic type. Itoyama d colleagues[32] reported the results of cytogenetic analysis of 50 cases ATLL and found aneuploidy and multiple breaks more frequently acute and lymphoma types. Multiple breaks and partial loss of omosomes correlated with shorter survival. The authors claim that e model of an oncogenic mechanism—activation of a proto-ogene by translocation of a T-cell receptor (TCR) gene—might be applicable to the main pathway of development of ATLL and at a multistep process of leukemogenesis is required.

In a study by Tsukasaki and associates,[33] 64 patients with ATLL re analyzed by using comparative genomic hybridization (CGH). e most frequent observations were gains at chromosomes 14q, 7q, d 3p and losses at chromosomes 6q and 13q. Chromosome imbal-es, losses, and gains were observed more frequently in acute or mphoma types. An increased number of chromosomal imbalances re associated with a shorter survival. Paired samples (i.e., samples ained at different sites from four patients) and sequential samples m 13 patients (from six during both chronic phase and acute crisis d from seven during both acute onset and relapse) were examined CGH and Southern blotting for HTLV-I. All but two paired mples showed differences on CGH assessment. Two chronic/crisis mples showed distinct results regarding both CGH and HTLV-I egration sites, suggesting clonal changes in ATLL at crisis. In 11 ients, the finding of identical HTLV-I sites and clonally related GH results suggested a common origin of sequential samples. In ntrast to chronic/crisis samples, CGH results with all acute/relapse mple pairs showed the presence of clonally related but not evolu-nal subclones at relapse. It was concluded that clonal diversity is mmon during progression of ATLL and that CGH alterations are ociated with clinical course.

ole of *p53* and Other Tumor Suppressor Genes

53 is a nuclear phosphoprotein that functions as a tumor suppressor ne. A loss of normally functioning *p53* through mutation or allelic s has been found in several kinds of malignant neoplasms. Muta-ns of the *p53* gene have also been found in some patients with TLL.[34,35] According to the study by Cesarman and coworkers,[35] no 53 mutations were detected in samples from 11 patients with the ronic type of ATLL, whereas 9 (28%) of 28 samples from patients th the acute type of ATLL exhibited p53 mutations. In one patient, tumor sample obtained during the chronic phase did not have a utation of the p53 gene, but the mutation was subsequently tected in a sample that was obtained at crisis. These results suggest t alterations of the p53 gene might contribute to disease progres-n in a fraction of patients with ATLL.

Other putative tumor suppressor genes, $p15^{INK4B}$ and $p16^{INK4A}$, re reported to be associated with ATLL.[36–38] Yamada and associ-s[37] reported that 28 (25%) of 114 patients with ATLL showed mozygous deletions of the *p15* and/or *p16* genes. These results rrelated well with the clinical subtypes of ATLL. In addition, the ients with deleted *p15* and/or *p16* genes showed significantly tter survival than did patients in whom both genes were preserved < 0.0001). Moreover, three of the five chronic-type patients who gressed to acute-type ATLL lost the *p16* gene alone or both genes their exacerbation phase. These results suggest that the deletions *p15* and/or *p16* genes play a key role in the disease progression of me patients with ATLL. Uchida and colleagues[38] found the point utation of the *p16* gene in 3 (7%) of 44 patients with ATLL. It is ggested that the *p16* gene is inactivated not only by homozygous letion, but also by point mutation.

ole of HTLV-I Provirus

veral investigators have analyzed the implications of the integration ttern of HTLV-I provirus in the disease progression of ATLL.[39,40] is known that the neoplastic cells of ATLL have one copy of complete HTLV-I provirus per cell in some patients (complete-type), while others have multiple complete copies of the virus per cell (multiple-type). The HTLV-I proviruses in the remaining patients do not have the complete genome but rather have a defective genome (defective-type). Tsukasaki and associates[40] found that the median survival times (MST) for patients were 7 months, 24 months, and 33 months for defective-type, complete-type, and multiple-type ATLL, respectively ($P = 0.006$). Among 52 sequentially examined patients, the HTLV-I integration patterns changed in four patients (8%). In three of these four, the rearrangements of the TCR-β gene changed concomitantly, suggesting the appearance of a new ATLL clone. The researchers concluded that the frequent clonal change of ATLL at crisis reflects the emergence of multiple premalignant clones in viral leukemogenesis.

Tamiya and coworkers[39] reported the presence of two types of defective virus. Among them, type 2 defective virus with the deletion that includes 5′ LTR was found more frequently in the acute and lymphoma types (39%, 21 of 54) than in the chronic type (6%, 1 of 18). It is postulated that the high frequency of the type 2 defective viruses is caused by the genetic instability of HTLV-I provirus and that this defective virus is selected because it escapes from the immune surveillance system in the host.

HTLV-I is an etiologic agent not only in ATLL, but also in the neurologic disorder known as tropical spastic paraparesis (TSP) or as HTLV-I-associated myelopathy (HAM).[41,42] In TSP/HAM, the HTLV-I provirus remains randomly integrated, whereas in ATLL, the provirus is monoclonally integrated.

EPIDEMIOLOGY OF HTLV-I AND ADULT T-CELL LEUKEMIA-LYMPHOMA

Southwestern Japan has the highest recorded prevalence of HTLV-I infection and the highest incidence of patients with ATLL in the world.[8,42–44] A high prevalence of HTLV-I is also found in the Caribbean islands (African), tropical Africa (African), South America (Mongoloid), and northern Oceania (Melanesian).[43–46] Many patients who have been diagnosed as having ATLL in Western countries are immigrants from the West Indies and tropical Africa. The world map of the distribution of HTLV-I and HTLV-II and the presumed routes of spread are shown in Figure 114-2.[44] The geographic clustering of HTLV-I carriers is suggested to be strongly associated with a high frequency of mother-to-child transmission of the virus under closed conditions in particular groups.[47]

It has been estimated that approximately 1.2 million HTLV-I-infected individuals reside in Japan, and the annual incidence of ATLL has been estimated to be approximately 700 in Japan.[8] The annual rate of ATLL development among HTLV-I carriers older than 40 years is estimated at 1.5 per 1000 in males and 0.5 per 1000 in females, and the cumulative risk of ATLL development among the HTLV-I carriers is estimated to be 2.5% to 5% over the course of a 70-year life span.[48]

In a national survey in Japan, the mean age of patients with ATLL has been estimated at 57.6 years, and this age appears to have increased over time.[8] It has been reported that the age of patients with ATLL in areas outside Japan is somewhat lower, with an overall mean age in the mid-forties.[49] In endemic areas, there is a marked increase in HTLV-I prevalence with age until age 70 years and an increased prevalence among females compared with males. Transmission occurs via sexual and blood-borne routes. A major reason for the increase in seroprevalence with age appears to be the decreasing prevalence of HTLV-I in the population over time, at least in Japan, where it has been most extensively studied. Yamaguchi and coworkers[50] reported that the HTLV-I carrier rates among blood donors in Japan had fallen since 1986 in all age groups under 50 years and in both genders. This decrease in HTLV-I carriers among younger blood donors might be explained by improvements in sanitation and general lifestyle changes in recent years. A shorter duration of breast-feeding,

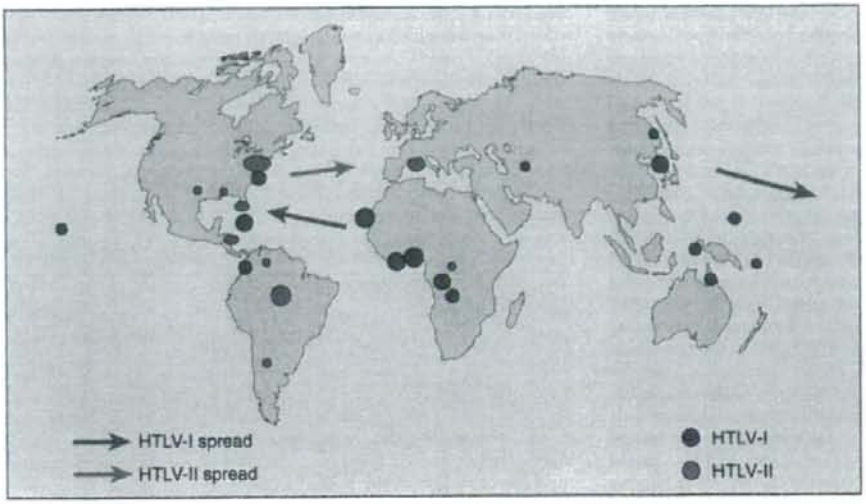

Figure 114-2 • World map of HTLV distribution and its presumed routes of spread. (From Blattner WA, Gallo RC: Epidemiology of HTLV-I and HTLV-II infection. In Takatsuki K [ed]: Adult T-cell Leukaemia. Oxford, UK, Oxford University Press, 1994, p 45. Prepared by Dr. Robert J Biggar, National Cancer Institute, USA.)

the increasing use of artificial feeding for babies, and decreasing family size are also likely to be factors for the recent decline in the vertical transmission rates of HTLV-I.[50] Overall, there is a slight male predominance of ATLL patients, the male-to-female ratio ranging from 1.1 to 1.5. This is in contrast to TSP and HAM, which affect females more frequently than males.

It has been shown that HTLV-I is transmitted by at least three routes:

1. Mother-to child-transmission, mainly by HTLV-I-positive lymphocytes in breast milk.[51]
2. Sexual transmission, more commonly from males to females.
3. Blood-borne transmission, including blood transfusions and sharing of needles by intravenous drug abusers.[52,53]

The first route is vertical transmission from mother to child via HTLV-I-positive lymphocytes in breast milk. The overall infection rate of HTLV-I in children by seropositive mothers has been estimated to be 10% to 30%. HTLV-I infection has also been reported in children who had not been breast-fed, however, which suggests the possibility of intrauterine or transvaginal infection. Several kinds of intervention trials are being conducted in HTLV-I-endemic areas in Japan, where seropositive pregnant women are advised not to breast-feed.[47]

The second route is transmission through sexual contact. Transmission of HTLV-I frequently occurs from male to female but rarely from female to male. HTLV-I has been isolated in semen. It appears likely that the risk of development of ATLL after HTLV-I infection by this route of transmission is not high.

To prevent HTLV-I transmission through blood transfusions, serologic screening of all blood donors for HTLV-I has been conducted in Japan since November 1986. Inaba and coworkers[54] reviewed the effectiveness of the donor screening in preventing transmission of HTLV-I through blood transfusion in Japan. Seroconversion was found in only 1 of 4672 transfused patients, but the donor was confirmed to be negative for anti-HTLV-I antibody and virus genome by nested polymerase chain reaction (PCR). A total of 23,323 red cell concentrates and 17,237 platelet concentrates were transfused to these 4672 patients. Therefore, the anti-HTLV-I prevalence in blood for transfusion after screening was estimated at 1 in 45,560 (0.0022%; the upper 95% confidence interval (CI) was 0.0080%). This study confirmed that the present donor screening program for HTLV-I can almost completely prevent virus transmission by transfusion in Japan. In contrast to red cell and platelet concentrates, fresh-frozen plasma and plasma fractions have never been shown to transmit HTLV-I.

From the viewpoint of the epidemiologic aspects of HTLV-I and ATLL, several points can be made in ATLL leukemogenesis:

- Viral infection alone is not adequate for the expression of the malignant phenotype.
- The timing and/or length of viral exposure is critical.
- The long latency period suggests that the disease progression is a multistep process. This is in contrast to TSP/HAM, which can occur with a shorter latency period, especially among recipients of blood transfusions.

CLINICAL MANIFESTATIONS

After HTLV-I was revealed to be associated with ATLL, it was found that ATLL shows a marked diversity in its clinical manifestations. ATLL cases have been subdivided into four distinct clinicopathologic entities: acute, lymphoma, chronic, and smoldering types. The recognition of the four clinical subtypes is important in understanding the natural history, clinical features, treatment strategy, and leukemogenesis of ATLL. On the basis of the nationwide survey of 854 patients with ATLL who were diagnosed between 1983 and 1987 in Japan, the Lymphoma Study Group proposed the diagnostic criteria of the four clinical subtypes (Table 114-1):[55]

1. The acute type shows a rapidly progressive clinical course and most of the characteristic features of ATLL: generalized lymphadenopathy, hepatomegaly, splenomegaly, skin involvement, hypercalcemia, and organ infiltration (lung, gastrointestinal tract, etc.). The symptoms and signs include abdominal pain, diarrhea, ascites, pleural effusion, cough, sputum, and chest x-ray abnormalities.
2. The smoldering type shows an indolent clinical course and only a small percentage of leukemic cells, but it also can include skin involvement.
3. The chronic type, with a high percentage of leukemic cells, is occasionally associated with skin involvement, lymphadenopathy, and hepatosplenomegaly and also shows an indolent clinical course.
4. The lymphoma type includes patients who present with the manifestations of non-Hodgkin's lymphoma (NHL) without circular-

Table 114-1 Diagnostic Criteria for Clinical Subtypes of Adult T-Cell Leukemia-Lymphoma

	Smoldering	Chronic	Lymphoma	Acute
Anti-HTLV-I antibody	+	+	+	+
Lymphocyte ($\times 10^3/\mu L$)	<4	≥4[†]	<4	*
Abnormal T lymphocytes	≥5%[§]	+[‡]	≤1%	+[‡]
Flower cells with T-cell marker	[†]	[†]	No	+
LDH	≤1.5 N	≤2 N	*	*
Corrected Ca^{2+} (mEq/L)	<5.5	<5.5	*	*
Histology-proven lymphadenopathy	No	*	+	*
Tumor lesion				
Skin and/or lung	*	*	*	*
Lymph node	No	*	Yes	*
Liver	No	*	*	*
Spleen	No	*	*	*
Central nervous system	No	*	*	*
Bone	No	No	*	*
Ascites	No	No	*	*
Pleural effusion	No	No	*	*
Gastrointestinal tract	No	No	*	*

HTLV-I, human T-lymphotropic virus type I; LDH, lactate dehydrogenase; N normal upper limit.
*No essential qualification except terms required for other subtype(s).
†Typical "flower cells" may be seen occasionally.
‡Accompanied by T lymphocytosis ($3.5 \times 10^3/\mu L$ or more).
§If abnormal T lymphocytes are less than 5% in peripheral blood, histologically proven tumor lesion is required.
‖Histologically proven skin and/or pulmonary lesion(s) is required if there are fewer than 5% abnormal T lymphocytes in peripheral blood.
From Shimoyama M, Members of the Lymphoma Study Group (1984–1987): Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma. Br J Haematol 1991;79:428.

ing malignant cells in the peripheral blood. When patients with ATLL are staged according to the Ann Arbor classification, most patients are categorized as stage IV, because leukemic cells are recognized even in clinically indolent forms such as the smoldering type and chronic type. Therefore, in ATLL, the clinical subtype is more important than the Ann Arbor stage for predicting prognosis and determining appropriate treatment strategies for individual patients.

ATLL, particularly the aggressive forms (acute and lymphoma types), has been found to infiltrate the stomach and the intestines in 29% and 25% of patients, respectively, at autopsy.[56] The involvement may be focal as an isolated gastric lesion or so diffuse as to involve the entire gastrointestinal tract. Extensive infiltration of the intestines can lead to moderate to severe diarrhea and malabsorption. Patients with ATLL suffer from a variety of abdominal symptoms (e.g., nausea, vomiting, abdominal fullness, and diarrhea), which might be attributable to infiltration by neoplastic cells, but because of the associated immunodeficiency, various opportunistic infections such as Strongyloidiasis can complicate cases.

Hepatic involvement of ATLL cells can be found in up to one fourth of patients with acute and lymphoma subtypes and not infrequently manifests with jaundice and hepatic transaminase elevations. Yamada and coworkers[57] examined 111 patients with acute-type or lymphoma-type ATLL and compared them with 106 patients with NHL other than ATLL. Among patients with ATLL, there were more frequent palpable hepatomegaly, higher total bilirubin, hepatic transaminase, LDH, and alkaline phosphatase values than among other NHL patients. Autopsy liver samples disclosed that the portal area was most frequently infiltrated with ATLL cells.

Pulmonary complications, which are common in ATLL, are due to leukemic infiltration in one half of patients and to infections with a variety of bacterial and opportunistic organisms in the other half.[58] Of 854 Japanese patients with ATLL, 26% had active infections at the time of diagnosis.[55] The incidence was highest among patients with the chronic and smoldering types (36%) and lower for patients with the acute (27%) and lymphoma (11%) subtypes. The infections that were encountered were bacterial (pneumonias, sepsis, and tuberculosis) in 43%, fungal in 31%, protozoal in 18%, and viral in 8% of patients with ATLL (Table 114-2). The immunodeficiency at presentation in ATLL can be exacerbated by the neutropenia that is produced by cytotoxic chemotherapy, leading to an extremely high risk of infection throughout the course of therapy. Infections are responsible for the patient's death in about half of the cases.

Central nervous system involvement occurs in approximately 10% of patients with ATLL. Teshima and associates[59] identified 15 instances of central nervous system involvement in 10 of 99 patients with ATLL. Leptomeningeal involvement was present in 9 of 10 patients, intracerebral infiltration was noted in 3, and the spinal cord was involved in 2. The initial symptoms included muscle weakness (47%), altered mental status (47%), paresthesias (40%), headache (33%), and urinary incontinence (27%). Signs included nuchal rigidity (33%) and cranial nerve palsies (13%). Hyponatremia secondary to the syndrome of inappropriate secretion of antidiuretic hormone was observed in four patients.

Table 114-2 Infectious Complications at Diagnosis in 818 Japanese Patients with Adult T-Cell Leukemia-Lymphoma

Infection	No. of Patients*				
	Acute	Lymphoma	Chronic	Smoldering	Total
Bacterial infection	(55)	(9 + 1)†	(25)	(4)	(93)
Pneumonia	35‡	1	14	4	54
Pyoderma	1	1	3	0	5
Septicemia	6	0	1	0	7
Tuberculosis	7	1	3	0	11
Other	6	6	4	0	16
Fungal infection§	(36 + 2)‡	(6)	(16)	(8)	(66)
Cutaneous	26	5‖	12	5	48
Oral	2	0	0	0	2
Esophageal	2	0	2	1	5
Pulmonary	5	1	1	0	7
Meningitis	1	0	1	2	4
Protozoal infection¶	(22)	(2)	(10)	(4)	(38)
Strongyloidiasis	13‡	2	5	1	21
Giardiasis	1	0	0	0	1
Pneumocystis carinii	8	0	5	3	16
Viral infection#	(13)	(0)	(3)	(0)	(16)
Herpes zoster	7	0	2	0	9
CMV pneumonia	3	0	0	0	3
Pneumonitis	2	0	1	0	3
Condyloma acuminatum	1	0	0	0	1
No infection**	339	139	98	29	605
Total	465	156	152	45	818

CMV, cytomegalovirus.
*Numbers in parentheses indicate total number of patients in each category.
†One patient had leprosy.
‡One patient each suffered from oral candidiasis.
§$P < 0.05$.
‖$P < 0.01$.
From Shimoyama M, Members of the Lymphoma Study Group (1984–1987): Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma. Br J Haematol 1991;79:42.

LABORATORY FINDINGS

Laboratory findings also depend on the clinical subtype of ATLL (see Table 114-1).[55] Leukocytosis is found among patients with the acute or chronic subtype at presentation, exhibiting characteristic atypical lymphoid cells with markedly lobated nuclei, termed *flower cells*. Although not all patients present with a leukemic feature, peripheral blood involvement develops in most patients at some time during the course of their disease. Most patients with the acute or lymphoma subtype of ATLL have elevated serum LDH levels.

The most striking laboratory finding in patients with ATLL is hypercalcemia, which was evident in 32% of Japanese patients with ATLL.[55] Multiple factors have been suggested to contribute to the development of hypercalcemia. Lytic bone lesions have been described in some patients; however, examinations of bone obtained at autopsy or from bone marrow biopsies usually reveal activated osteoclasts with increased bone resorption; infiltrating neoplastic T cells are rarely found. Patients with ATLL have low phosphate levels, hypercalciuria, high levels of nephrogenous cyclic adenosine monophosphate, and low levels of 1,25-dihydroxyvitamin D. This pattern suggests the presence of humoral hypercalcemia of malignancy, which was found to be secondary to the production of a parathyroid hormone (PTH)-like molecule by malignant cells. HTLV-I-infected cells were found to produce a protein with PTH-like activity, such as PTH-related peptide.[60–62] Another suggested contributor to hypercalcemia in patients with ATLL is cytokine production by the tumor cells. HTLV-I-infected cell lines and fresh ATLL cells from hypercalcemic patients produce TNF-α, TNF-β, IL-1α, and IL-1β. Each of these cytokines can enhance osteoclast activity and bone-resorbing activity in animal models. Ishibashi and colleagues[63] demonstrated elevated serum levels of TNF-β in seven of eight patients with ATLL who had complications of hypercalcemia and in none of 28 patients with ATLL who had normal serum calcium levels.

Nosaka and coworkers[64] analyzed the expression of various genes that were suggested to regulate serum calcium levels in ATLL and reported that the overexpression of the receptor activator of NF-κB (RANK) ligand gene correlated with hypercalcemia. ATLL cells from patients with hypercalcemia, which highly expressed the transcripts of the RANK ligand (RANKL) gene, induced the differentiation of human hematopoietic precursor cells (HPCs) into osteoclasts in vitro in the presence of macrophage colony-stimulating factor. In contrast, ATLL cells from patients without hypercalcemia did not induce such

differentiation, suggesting that the induction of the differentiation correlated with the expression of the RANKL gene in ATLL cells. Cell differentiation was suppressed by osteoprotegerin/Fc, an inhibitor of RANKL, suggesting that such differentiation occurred through the RANK-RANKL pathway. In addition, direct contact between ATLL cells and hematopoietic precursor cells was essential for the differentiation, suggesting that membrane-bound RANKL rather than the soluble form plays a role in this process. The authors claimed that ATLL cells induce the differentiation of hematopoietic precursor cells to osteoclasts through RANKL expressed on their surface, in cooperation with macrophage colony-stimulating factor, and that they ultimately cause hypercalcemia. The etiology of hypercalcemia in ATLL is likely to be multifactorial and in individual patients is probably due to some combination of the factors just described.

Elevated serum levels of soluble interleukin-2 receptor in patients with ATLL, especially in those with the acute or lymphoma subtype, have been noted in several studies.[65] The serum level of soluble interleukin-2 receptor is suggested to be one of the useful markers for evaluating the clinical aggressiveness of the disease and for monitoring the response to therapy in patients with ATLL.

Histopathology

The circulating cells in the peripheral blood have markedly polylobated nuclei with homogeneous and condensed chromatin, small or absent nucleoli, and agranular and basophilic cytoplasm—the so-called flower cells that are characteristic of ATLL (Fig. 114-3).[55] A considerable diversity of morphology among ATLL cells has been recognized, however. Tsukasaki and associates[66] investigated the morphology of ATLL cells in 36 acute cases and 14 chronic cases. Chronic lymphocytic leukemia–like morphology with round nuclei was more frequent in the chronic type than in the acute type. In contrast, unusual morphology (lymphoblastic, vacuolated, granular pleomorphic, or large cells) was more frequent in the acute type than in the chronic type.

The swollen lymph nodes in patients with ATLL show diffuse NHL of various histologic subtypes, including pleomorphic, large cell, mixed cell, or medium-sized cell types.[67,68] Figure 114-4 shows the histology of a biopsied swollen lymph node from a patient with lymphoma-type ATLL. The pleomorphic pattern (i.e., a mixture of various-sized lymphoma cells from small cells to giant cells) and nuclear polymorphism are recognized. Lymph nodes from some patients in the incipient or early neoplastic phase of ATLL histologically resemble those that are found in Hodgkin's lymphoma.[69–71]

ATLL cells frequently involve the skin. Generalized nodular or papulonodular eruptions, as shown in Figure 114-5A, are common; however, tumorous lesions are also recognized in some patients. Erythematous plaque formation and sometimes nodular tumors are other cutaneous manifestations.[71,72] Histologically, diffuse or patchy infiltration of atypical lymphoid cells—usually small or medium in size with polymorphic nuclear contours in the upper dermis, sometimes with an intraepidermal infiltration—is noted (Fig. 114-5B). Large nuclear cells with highly irregular or cerebriform features are intermingled in some cases.

One of the difficult issues in the diagnosis of ATLL is its relationship with other peripheral T-cell malignancies that are not associated with HTLV-I. The clinical diagnosis of ATLL is suspected by the unique combination of its clinical and pathologic features. One of the T-cell malignancies that is likely to be confused with ATLL is mycosis fungoides/Sézary syndrome (MF/SS). Because cutaneous involvement is frequent in ATLL, the differentiation of smoldering-type ATLL from MF/SS is often difficult on the basis of the clinical manifestations alone. In the differential diagnosis of ATLL and other peripheral T-cell malignancies, HTLV-I serology and the molecular detection of the monoclonal integration of HTLV-I proviral DNA are important. Various kinds of serologic assays have been used, including the immunofluorescence assay, the particle agglutination assay, the enzyme-linked immunosorbent assay (ELISA), and the Western blot assay. In general, a particle agglutination assay or ELISA is useful as a screening test, and Western blotting is used for the confirmation of the presence of serum antibody to HTLV-I. As shown in Figure 114-6, the demonstration of the monoclonal integration of HTLV-I proviral DNA by Southern blot analysis can lead to a definite diagnosis of ATLL.

Several studies have reported the presence of seronegative HTLV-I carriers, and some HTLV-I carriers have been reported to be negative for serum anti-HTLV-I antibodies against viral structural proteins on screening examinations.[73] Kinoshita and associates[74] examined peripheral blood mononuclear cells from 209 healthy subjects living in an HTLV-I-endemic district in Japan for HTLV-I provirus, using PCR. A total of 76 subjects were positive for the provirus and 133 were negative, showing a close correlation with the results of the previously mentioned assays for anti-HTLV-I serum antibodies. None of the seronegative subjects reacted positively in PCR analysis. Infrequent HTLV-I infection among seronegative subjects in Japan was also suggested by the finding that the screening of blood donors for serum HTLV-I antibodies by the PA assay has reduced markedly the risk of HTLV-I transmission by blood transfusions.[75] Furthermore, by using PCR analysis, the absence of seronegative HTLV-I carriers among blood donors and healthy junior high school students in Japan was confirmed.[76] These observations suggest that seronegative HTLV-I carriers are extremely rare, although the possibility of their existence remains.

In Western countries, it has been reported that the HTLV-I viral genome was detected in the genomic DNA from patients with MF/SS, and a causal relation between HTLV-I and MF/SS was proposed.[77,78] An opposite conclusion was reached in a Japanese study; using PCR with four sets of primers (including *gag*, *pol*, *env*, and *pX* regions of HTLV-I), Kikuchi and colleagues[79] investigated both fresh and cultured T cells (128 specimens) derived from 50 Japanese patients with CTCL. In their study, none of the 128 DNA samples revealed positive results for HTLV-I. They concluded that CTCL, which does not include HTLV-I, is present in Japan. The absence of a correlation between HTLV-I and CTCL was confirmed by an international cooperative study reported by Bazarbachi and coworkers.[80] These researchers analyzed 128 patients (85 with MF, 28 with SS, 5 with Sézary cell leukemia, 4 with lymphomatoid papulosis, and 5 with unspecified CTCL) originating from Europe (France, Spain, United Kingdom, or Portugal) or from the United States (California) for the presence of HTLV-I infection markers, using a serologic analysis for antibody to HTLV-I, a reverse transcriptase assay, and a molecular analysis with PCR-amplified specimens. The results of this international study suggest that MF and SS are not associated with HTLV-I infection.

HTLV-I can infect lymphoid cells of different cell lineages in vitro, but the neoplastic cells in the great majority of ATLL cases exhibit the phenotype of mature CD4-positive T cells.[81] Malignant cells from the peripheral blood or from involved lymph nodes express CD2, CD3, CD4, CD5, the αβ-chains of the TCR, CD25 (IL-2Rα), CD45, CD29, and HLA-DR.[82,83] It is known that the expression of the CD3/TCR complex is decreased in ATLL cells.[84] Most ATLL cells lack CD7. Considerable phenotypic heterogeneity has been found in the neoplastic cells of ATLL. Although the most common phenotype is CD4-positive/CD8-negative, some patients with ATL exhibit a CD4-positive/CD8-positive, CD4-negative/CD8-positive, or CD4-negative/CD8-negative phenotype. In addition, some patients with ATLL show phenotypic changes throughout the course of their disease. As regulatory T cells (Treg cells) express CD4-positive and CD25-positive molecules and possess potent immune response suppressive activity, several investigators analyzed a possible link between ATLL cells and Treg cells and found that forkhead/winged helix transcription factor (FoxP3), a

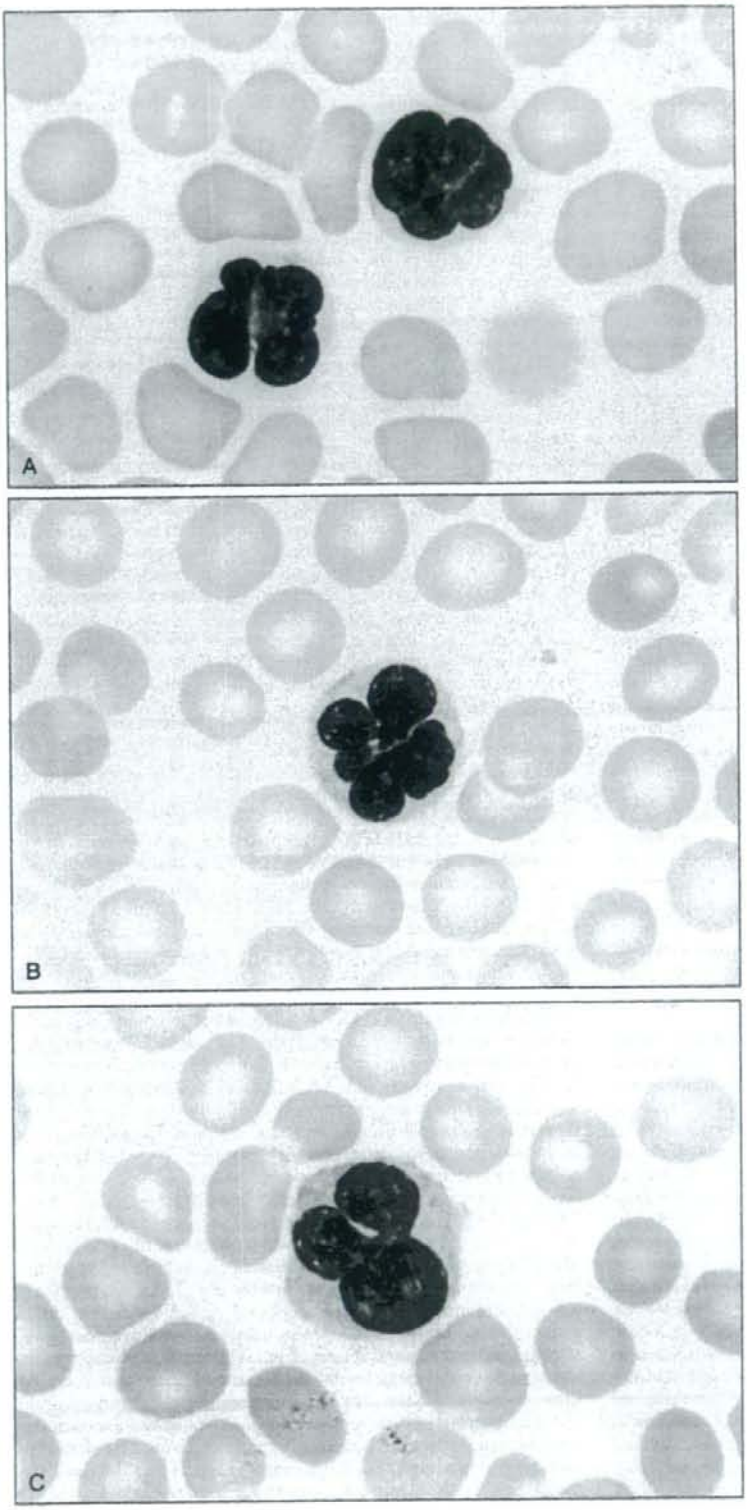

Figure 114-3 • **A–C,** Leukemic cells (the so-called flower cells) showing characteristic polymorphic nuclei in a peripheral blood smear from a patient with acute-type ATLL.