| | | | | | |
|---|---|---|---|---|---|
| Okada A, Yamasaki S, Koga T, Kawashiri SY, Tamai M, Origuchi T, Nakamura H, Eguchi K, Kawakami A. | Adipogenesis of the mesenchymal stromal cells and bone oedema in rheumatoid arthritis. | Clin Exp Rheumatol. | 30(3) | 332-7 | 2012 |
| Tamai M, Kawakami A, Uetani M, Fukushima A, Arima K, Fujikawa K, Iwamoto N, Aramaki T, Kamachi M, Nakamura H, Ida H, Origuchi T, Aoyagi K, Eguchi K. | Magnetic resonance imaging (MRI) detection of synovitis and bone lesions of the wrists and finger joints in early-stage rheumatoid arthritis: comparison of the accuracy of plain MRI-based findings and gadolinium-diethylenetriamine pentaacetic acid-enhanced MRI-based findings. | Mod Rheumatol | 22(5) | 654-8 | 2012 |
| Kita J, Tamai M, Arima K, Nakashima Y, Suzuki T, Kawashiri SY, Iwamoto N, Okada A, Koga T, Yamasaki S, Nakamura H, Origuchi T, Ida H, Aoyagi K, Uetani M, Eguchi K, Kawakami A | Treatment discontinuation in patients with very early rheumatoid arthritis in sustained simplified disease activity index remission after synthetic disease-modifying anti-rheumatic drug administration. | Mod Rheumatol | 22(3) | 346-52 | 2012 |

| | | | | | |
|---|---|---|---|---|---|
| Kita J, Tamai M, Arima K, Nakashima Y, Suzuki T, Kawashiri SY, Okada A, KogaT, Yamasaki S, Nakamura H, Origuchi T, Aramaki T, Nakashima M, Fujikawa K, Tsukada T, Ida H, Aoyagi K, Uetani M, Eguchi K, Kawakami A. | Delayed treatment with tumor necrosis factor inhibitors in incomplete responders to synthetic disease-modifying anti-rheumatic drugs shows an excellent effect in patients with very early rheumatoid arthritis with poor prognosis factors. | Mod Rheumatol | 22(2) | 195-201 | 2012 |
| Ichinose K, Origuchi T, Kawashiri SY, Iwamoto N, Fujikawa K, Aramaki T, Kamachi M, Arima K, Tamai M, Nakamura H, Ida H, Kawakami A, Eguchi K. | Long-term follow-up of adalimumab monotherapy for rheumatoid arthritis in Japanese patients: a report of six cases. | Rheumatol Int | 32(2) | 483-7 | 2012 |
| 屋良さとみ, 山里代利子, 熱海恵理子, 玉寄真紀, 藤田次郎 | 気道感染症 HTLV-1 関連気管支病変 | 日本胸部臨床 | 71 | S132-S138 | 2012 |
| 平田哲生, 藤田次郎 | 糞線虫過剰感染症候群 | 内科 | 109(6) | 1437-1438 | 2012 |
| 天野正宏, 瀬戸山充 | 皮膚リンパ腫と鑑別すべき疾患、偽リンパ腫を中心に | 日本皮膚科学会雑誌 | 122(13) | 3165-3168 | 2012 |
| Kamoi K, Mochizuki M | HTLV-1 uveitis. | Front Microbiol | 3 | 270 | 2012 |

| Kamoi K, Mochizuki M | HTLV infection and the eye. | Curr Opin Ophthalmol | 23(6) | 557-561 | 2012 |
|---|---|---|---|---|---|
| Ohguro N, Sonoda K, Takeuchi M, Matsumura M, Mochizuki M | The 2009 prospective multi-center epidemiologic survey of uveitis in Japan. | Jpn J Ophthalmol | 56(5) | 432-435 | 2012 |
| Takenouchi H, Umeki K, Sasaki D, Yamamoto I, Nomura H, Takajo I, Ueno S, Umekita K, Kamihira S, Morishita K, Okayama A | Defective human T-lymphotropic virus type 1 provirus in asymptomatic carriers. | Int J Cancer | 128(6) | 1335-43 | 2011 |
| 梅北邦彦, 日高利彦, 岡山昭彦 | 話題4. IL-6 阻害薬と感染症のマスキングとは？ | 臨床検査. 増刊号 ここまでわかった自己免疫疾患 | 55(11) | 1367-70 | 2011 |
| 高城一郎, 岡山昭彦 | 特集(1)：HTLV-1 感染の検査と臨床　2. HTLV-1 感染の疫学 | 医療と検査機器・試薬 別冊 機器・試薬 | 34(4) | 447-452 | 2011 |
| Koga T, Yamasaki S, Migita K, Kita J, Okada A, Kawashiri S, Iwamoto N, Tamai M, Arima K, Origuchi T, Nakamura H, Osaki M, Tsurumoto T, Shindo H, Eguchi K, Kawakami A | Post-transcriptional regulation of IL-6 production by Zc3h12a in fibroblast-like synovial cells. | Clin Exp Rheumatol | 29 (6) | 906-12 | 2011 |

| Ohyama K, Ueki, Kawakami A, Tamai M, Osaki M, Kamihira S, Nakashima K, Kuroda N | Immune complexome analysis of serum and its application in screening for immune complex antigens in rheumatoid arthritis. | Clin Chem. | 57 (6) | 905-909 | 2011 |
|---|---|---|---|---|---|
| Kawashiri SY, Kawakami A, Iwamoto N, Fujikawa K, Satoh K, Tamai M, Nakamura H, Okada A, Koga T, Yamasaki S, Ida H, Origuchi T, Eguchi K | The power Doppler ultrasonography score from 24 synovial sites or 6 simplified synovial sites, including the metacarpophalangeal joints, reflects the clinical disease activity and level of serum biomarkers in patients with rheumatoid arthritis. | Rheumatology | 50 (5) | 962-965 | 2011 |
| Koga T, Okada A, Kawashiri S, Kita J, Suzuki T, Nakashima Y, Tamai M, Satoh K, Origuchi T, Iwamoto N, Yamasaki S, Nakamura H, Migita K, Ida H, Ueki Y, Eguchi K, Kawakami A | Soluble urokinase plasminogen activator receptor as a useful biomarker to predict the response to adalimumab in patients with rheumatoid arthritis in a Japanese population. | Clin Exp Rheumatol | 29 (5) | 811-815 | 2011 |
| Kawashiri SY, Kawakami A, Okada A, Koga T, Tamai M, Yamasaki S, Nakamura H, Origuchi T, Ida H, Eguchi K | CD4+CD25(high)CD127 (low/-) Treg cell frequency from peripheral blood correlates with disease activity in patients with rheumatoid arthritis. | J Rheumatol | 38 (12) | 2517-21 | 2011 |

| Li H, Zhou Y, Fan F, Zhang Y, Li X, Yu H, Zhao L, Yi X, He G, Fujita J, Jiang D. | Effect of azithromycin on patients with diffuse panbronchiolitis: retrospective study of 51 cases. | Intern Med | 50(16) | 1663-9 | 2011 |
|---|---|---|---|---|---|
| Nakayama Y, Ishikawa C, Tamaki K, Senba M, Fujita J, Mori N | Interleukin-1 alpha produced by human T-cell leukaemia virus type I-infected T cells induces intercellular adhesion molecule-1 expression on lung epithelial cells. | J Med Microbiol | 60 (Pt12) | 1750-61 | 2011 |
| Amano M, Marutsuka K, Sugimoto T, Todaka T, Setoyama M | Epstein-barr virus-associated primary central nervous system lymphoma in a patient with adult T-cell leukemia/lymphoma. | The Journal of Dermatology | 38(6) | 575- 80 | 2011 |
| Amano M, Setoyama M, Annika Grant, Francisco A Kerdel | Human T-lymphotropic Virus 1(HTLV-1) infection - dermatological implications. | International Journal of Dermatology | 50(8) | 915- 20 | 2011 |

# Ⅲ. 研究成果の刊行物・別刷

# 2 成人T細胞白血病/リンパ腫

天野 正宏

ヒトT細胞白血病ウイルス1型（HTLV-1）によるT細胞の悪性新生物である．2008年のWHO分類（第4版）では，菌状息肉症（MF）やSézary症候群（SS）とともに，皮膚T細胞・NK細胞リンパ腫のなかに分類されている．母乳を介した感染が90%以上を占め，HTLV-1の感染後，約50～60年間という長い潜伏期間の後，1～5%が成人T細胞白血病/リンパ腫（adult T-cell leukemia/lymphoma：ATLL）を発症するとされる．HTLV-1の高度浸淫地域，すなわち日本南西部，カリブ海諸島，南アメリカ，中央アフリカの諸地域では，ATLLは一種の風土病とされてきた．わが国南西部では20数年前から抗HTLV-1抗体陽性の妊婦に対し母乳中止や制限措置が功を奏し，抗HTLV-1抗体陽性者が減少してきた．しかし最近，人口移動などにより抗HTLV-1抗体陽性者が全国的な広がりをみせているとの報告を受け，2010年，政府および厚生労働省が全国的に妊婦に全例抗HTLV-1抗体検査を義務付け，またATLLやHTLV-1関連脊髄症（HAM）を含めたHTLV-1関連疾患の治療および研究に予算をつけている．

ATLLは末梢血液像（特に異常リンパ球%），血清LDH（lactate dehydrogenase）値，血清補正カルシウム値，臓器病変の有無などを基に，急性型，リンパ腫型，慢性型，くすぶり型に分けられる．急性型やリンパ腫型ATLLは治療抵抗性を示し，極めて予後不良である．これに対し，慢性型やくすぶり型ATLLは慢性に経過するが，急性転化することもある．ATLLのなかには末梢血液に異常リンパ球もなく，またリンパ節浸潤や臓器病変を伴わず，皮膚病変（特異疹）だけを認めることもあり，急性型やリンパ腫型ATLLと同様に予後不良であり，皮膚型ATLLの存在が提唱されている．筆者らの皮膚型ATLLの診断基準（私案）は，確定診断時に，①ATLLの特異疹があり，②リンパ球数＜4,000/μL，③皮膚以外に臓器浸潤を認めない，である．

## 診断と検査

ATLLの診断では，まず患者がどの臨床病型にあるかが重要である．なぜなら病型によって予後が決定するからである．急性型やリンパ腫型ATLLの生存期間は2週間～1年未満である．慢性型やくすぶり型ATLLでは，より遷延性の臨床経過をとり長期間生存することもあるが，急性型ATLLへ転化することがあるので，注意深い経過観察が必要である．

診断には，まず採血にて抗HTLV-1抗体の有無をチェックし，陽性であれば末梢血液検査（異常リンパ球，特に花細胞の有無が重要），生化学検査（特にLDH値，補正カルシウム値）を行う．次にCT，MRIやPET-CTなどの画像診断を用いて，深部のリンパ節や内臓浸潤の有無を検査する．

ATLLを発症すると，その約半数にATLL細胞の皮膚への浸潤，すなわち特異疹を合併してくる．特異疹で最も多いのが結節または腫瘤（33%）であり，次に全身性に生じる丘疹（22%）または紅斑（19%）と続く．慢性型やくすぶり型ATLLでは高頻度に皮膚病変を伴い，また，くすぶり型では末梢血中の異常リンパ球が少数か欠如している場合は，臨床的にMFと鑑別が困難な場合がある．特異疹では病理組織学的に，多型または分葉化した核を持つ中型～大型のT細胞が表在性またはびまん性に浸潤し，しばしば著明な表皮向性を示す．くすぶり型ATLLでは，真皮への浸潤はまばらで，あってもわずかに異型細胞を認めるだけの場合もある．腫瘍性T細胞は$CD3^+$，$CD4^+$，$CD8^-$の表現型を呈し，CD25は高頻度に陽性である．病理組織像でMFと鑑別できない場合もある．T細胞受容体はATLLもMFやSSもクローナルに再構成を認めるため鑑別にはならないが，ATLLではクローナル

> **役に立つ豆知識**
>
> わが国で開発された抗CCR4モノクローナル抗体療法はATLLに対する治験が終了し，2012年2月1日に厚生労働省がATLLの治療薬として承認しており，臨床での今後の使用が見込まれる．適応は化学療法後に再発または再燃した抗CCR4抗体陽性ATLLである．治験においても特異疹の改善が認められており，今後，期待される薬剤である．

天野正宏：成人T細胞白血病/リンパ腫.皮膚疾患最新の治療2013-2014.瀧川雅浩,渡辺晋一.南江堂,223-224（2013）



なHTLV-1プロウイルスの組み込みが認められ，鑑別に有用である．また，表在リンパ節が腫脹している場合は，生検を行いATLL細胞の浸潤があるかどうかを確認する必要がある．

LDH値と可溶性IL-2R値はATLLの病勢をみる指標として重要である．急性転化し日和見感染症の合併が疑われる場合，β-Dグルカンは真菌感染症やニューモシスチス肺炎の有無や治療効果判定の指標となる．また，サイトメガロウイルス感染症が疑われる場合，血中サイトメガロウイルス抗原(C7-HRP)を測定すべきである．

## 治療の一般方針

皮膚病変を主体とする慢性型，くすぶり型や皮膚型ATLLでは，MFと同様に皮膚を標的とした局所療法(skin-directed therapy：SDT)が選択される．しかしSDTにより，必ずしもATLLの生命予後が改善されるわけではない．特異疹が紅斑や丘疹の場合，SDTとして副腎皮質ステロイド，抗癌薬の外用などが用いられるが，結節や腫瘤には効果が少なく，放射線(特に電子線)療法が選択される．特異疹が結節や腫瘤の場合．経過中に急性転化を生じ予後不良とされ，SDTに終始することなく，身体所見，採血，画像診断を含め病勢の悪化がないか注意深く観察し，急性転化の徴候あれば，単剤の化学療法薬を中心とした全身療法(systemic therapy：ST)へ切り替えていく必要がある．また，急性転化した場合は，後述の急性型，リンパ腫型ATLLと同様のSTが必要であり，前もって血液内科医と連携をとっておくことが肝要である．非特異疹として動物，真菌，細菌，ウイルスなどによる感染症を伴うことが多いので，感染症対策も必要である．

急性型，リンパ腫型ATLLでは，血液内科医と連携をとり多剤併用化学療法や同種造血幹細胞移植を中心としたSTが必要である．特に急性型ATLLでは高カルシウム血症を合併するので，補液，利尿薬，カルシトニン製剤，ビスホスホネート製剤などによりカルシウム値を下げると同時に，多剤併用化学療法を行う．さらに急性型ATLLでは，細胞性免疫の低下によるサイトメガロウイルス感染症やニューモシスチス肺炎などの真菌感染症を合併するため，抗ウイルス薬，抗真菌薬，ST合剤などの投与を併用する．

## 処方例

**a．局所療法(SDT)**

＜特異疹が紅斑，丘疹や局面の場合＞

①アンテベート軟膏：1日2回，外用

②紫外線療法(PUVA療法，ナローバンドUVB療法)：文献的には報告はあるが，筆者らは悪化した症例の経験があり施行していない．

③0.2% ACNUローション(ニドラン注)：1日2回，外用(保険適用外)．院内製剤として作成している．

＜特異疹が結節，腫瘤の場合＞

①ケナコルト-A：1%キシロカインで2～5倍に希釈し，結節や腫瘤に局注

②単発～数個の腫瘤であれば，電子線照射，計30～40 Gy程度

**b．全身療法(ST)**

①ラステット：50mg，分1～2，21日間内服し，1～2週間休薬する．これを1クールとして繰り返す．

②ペラゾリン細粒：1,600 mg，分1～2．5日間連続内服し，2～3週間休薬する．これを1クールとして繰り返す．

③プレドニゾロン：10～20 mg，分1～2，ラステットやペラゾリンの副作用予防として併用，また単独でも抗腫瘍効果が期待できる．

④(ニューモシスチス肺炎予防のため)バクタ：2～4錠，分2，週2回，連日内服

⑤(真菌症予防のため)ファンギゾンシロップ：24 mL，蒸留水などで500 mLに希釈し，毎食後に含漱

＜急性型，リンパ腫型ATLLの場合＞

血液内科医と連携をとり，多剤併用化学療法(modified LSG-15療法など)，同種造血幹細胞移植[年齢や状態により，骨髄破壊的あるいは骨髄非破壊的移植(いわゆるミニ移植)を選択]を行う．特に後者は，移植医療の専門医がいる施設でないと施行できない．

## 生活指導

適度な睡眠をとり，規則的なバランスのとれた食事を摂ること，疲労を蓄積しないこと，また精神的ストレスを避け，精神的に安定した状態を保つことなどを指導している．

uicc
global cancer control

IJC
International Journal of Cancer

# Proviral loads of human T-lymphotropic virus Type 1 in asymptomatic carriers with different infection routes

Shiro Ueno[1], Kazumi Umeki[1], Ichiro Takajo[1], Yasuhiro Nagatomo[1], Norio Kusumoto[1], Kunihiko Umekita[1], Kazuhiro Morishita[2] and Akihiko Okayama[1]

[1] Department of Rheumatology, Infectious Diseases and Laboratory Medicine, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan
[2] Division of Tumor and Cellular Biochemistry, Department of Medical Sciences, University of Miyazaki, Miyazaki, Japan

**High human T-lymphotropic virus Type 1 (HTLV-1) proviral DNA load (PVL) has been reported to be one risk factor for the development of adult T-cell leukemia/lymphoma (ATL). ATL is also believed to develop in HTLV-1 carriers who acquire infection perinatally. ATL cells have been reported to frequently harbor defective provirus. In our study, PVLs for three different regions of HTLV-1 provirus (5′LTR-*gag*, *gag* and *pX*) were measured in 309 asymptomatic carriers with different infection routes. PVLs for the *pX* region in 21 asymptomatic carriers with maternal infection was significantly higher than in 24 carriers with spousal infection. Among 161 carriers with relatively high *pX* PVLs (equal to or greater than 1 copy per 100 peripheral blood mononuclear cells), 26 carriers (16%) had low *gag* PVL/*pX* PVL (less than 0.5) and four (2%) had low 5′LTR-*gag* PVL/*pX* PVL (less than 0.5). Low *gag* PVL/*pX* PVL ratio, which reflects deficiency and/or polymorphism of HTLV-1 proviral DNA sequences for the *gag* region, was also associated with maternal infection. These data suggest that HTLV-1 carriers with maternal infection tend to have high PVLs, which may be related to provirus with deficiency and/or the polymorphism of proviral DNA sequences. In addition, there is a possibility that this ratio may be used as a tool to differentiate the infection routes of asymptomatic HTLV-1 carriers, which supports the need for a large scale study.**

Human T-lymphotropic virus Type 1 (HTLV-1) is the causative agent of adult T-cell leukemia/lymphoma (ATL) and a progressive neurological disease known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[1–4] Major routes of HTLV-1 infection have been reported as mother to child infection at infancy, sexual contact between spouses and blood transfusion.[5–7] The majority of HTLV-1 carriers are asymptomatic, and only a fraction of carriers develop ATL after a long latent period.[8,9] It has been reported that approximately 4% of HTLV-1 carriers develop ATL eventually.[10] Studies of the mothers of patients with ATL have reported most of them to be HTLV-1 carriers.[11,12] Therefore, ATL is believed to develop in HTLV-1 carriers who acquire infection perinatally. However, there has been no method of identifying the infection route of HTLV-1 positive individuals without information on family HTLV-1 status.

When an individual is infected by HTLV-1, the virus randomly integrates into the genome of affected T-cells in the form of provirus.[13] HTLV-1 infection drives the proliferation of T-cells, leading to the clonal expansion of HTLV-1 infected cells.[14–16] Recently, it was reported that HTLV-1 clonal expansion *in vivo* is favored by orientation of the provirus in the same sense as the nearest host gene.[17] We have reported that the clonality of HTLV-1 infected cells in adult seroconverters who were newly infected from HTLV-1 carrier spouses is more heterogeneous and less stable than that of long-term carriers who acquired infection from their mothers at infancy.[18] The selective maintenance of certain clones is supposed in the latter. Recently, we reported that clonal expansion of HTLV-1 infected cells was found in a certain population of asymptomatic carriers and that these carriers had high proviral DNA loads (PVLs).[19] High PVLs have been reported to be a risk factor for developing ATL.[20,21] In another study, we analyzed the PVLs of 13 pairs of HTLV-1 seroconverters and their spouses.[22] Although seroconverters and their spouses shared the same HTLV-1, PVLs in both individuals in a couple were not always equivalent. These findings suggested that host-related factors play an important role to determining the PVL in each carrier. However, it was

**Key words:** HTLV-1, defective virus, infection route, proviral DNA loads
**Abbreviations:** ATL: adult T-cell leukemia/lymphoma; HTLV-1: human T-lymphotropic virus type 1, LTR: long-terminal repeat, PBMCs: peripheral blood mononuclear cells, PCR: polymerase chain reaction; PVLs: proviral DNA loads
**Grant sponsors:** Ministry of Education, Science, Sports and Culture, Japan, Miyazaki Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, JST
**DOI:** 10.1002/ijc.26289
**History:** Received 22 Feb 2011; Accepted 16 Jun 2011; Online 21 Jul 2011
**Correspondence to:** Akihiko Okayama, Department of Rheumatology, Infectious Diseases and Laboratory Medicine, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan, Tel.: 81-985-85-7284
Fax: 81-985-85-4709, E-mail: okayama@med.miyazaki-u.ac.jp

not clear in that study whether HTLV-1 carriers who acquired infection from their mothers at infancy have more PVLs than the carriers who acquired infection from their spouses in adulthood.

Defective provirus has frequently been detectable in patients with ATL.[23–27] The complete HTLV-1 provirus is approximately 9 kb and contains the coding regions for core protein (*gag*), protease (*pro*), polymerase (*pol*), envelope protein (*env*), regulatory proteins, such as Tax and Rex, and some accessory molecules between 5′ and 3′ long-terminal repeats (LTRs).[8,28] Tamiya *et al.*[23] reported two types of genome deletion in defective provirus. One form retains both LTRs and lacks internal sequences, such as the *gag* and *pol* regions. The other form has the 3′ LTR, and the 5′ LTR and its flanking internal sequences are preferentially deleted. HTLV-1 infected cells harboring the latter defective virus were frequently found in patients with ATL.[26] Both types of defective provirus were suspected of being harbored by the clonally expanded HTLV-1 infected cells in asymptomatic carriers.[19] The polymorphism of the proviral genome was also found in asymptomatic carriers in that study; however, we could not show how commonly the deficiency or polymorphism of the proviral genome was detectable.

These questions prompted us to investigate HTLV-1 PVLs in asymptomatic carriers with different infection routes. In addition, to clarify whether the defective provirus and/or polymorphism of the proviral genome affected PVLs, we tested PVLs for three different regions (5′LTR-*gag*, *gag* and *pX*) of provirus in each individual and compared them among the carriers with different infection routes in our study.



## Material and Methods

### Samples

Samples of peripheral blood mononuclear cells (PBMCs) were obtained from 309 HTLV-1 carriers (103 men and 206 women, median age: 67 years), who had no symptoms or laboratory data suggesting HTLV-1 related disease, in the Miyazaki Cohort Study.[29] Infection routes were investigated by family HTLV-1 status and history of HTLV-1 seroconversion.[18,22] An HTLV-1 carrier with HTLV-1 positive mother/HTLV-1 negative spouse or with HTLV-1 positive siblings/HTLV-1 negative spouse or with HTLV-1 seroconverter was defined as infected by his/her mother. An HTLV-1 carrier who was a HTLV-1 seroconverter with HTLV-1 positive spouse or with HTLV-1 negative mother/HTLV-1 positive spouse was defined as infected by his/her spouse. Carriers with history of blood transfusion were excluded from the analysis of family status. As a result, 21 and 24 carriers were defined as infected by their mothers and by their spouses, respectively. Infection routes could not be determined in 264 carriers. Informed consent was obtained from the study participants and the study protocol was approved by the institutional review board at University of Miyazaki.

### Real-time polymerase chain reaction

PVLs for three different proviral regions (5′LTR-*gag*, *gag* and *pX*) were determined by real-time polymerase chain reaction (PCR) using Light Cycler 2.0 (Roche Diagnostics, Mannheim, Germany). Genomic DNA was isolated from PBMCs of asymptomatic HTLV-1 carriers by sodium dodecyl sulfate-proteinase K digestion, followed by phenol-chloroform extraction and ethanol precipitation. Approximately 100 ng genomic DNA was used as the template. The nucleotide position number of HTLV-1 provirus was according to Seiki *et al.*[30] (accession no. J02029). The primers and probes for real-time PCR were designed to minimize the differences of the melting points 5′LTR-*gag*, *gag* and *pX* and were as follows: 5′LTR-*gag*: the forward primer (5′LTR-SDS-F 5′-AAGTACCGGCGACTCCGTTG-3′: positions 700–719), the reverse primer (HTLV-gag-LTR-R2 5′-GGCTAGCGCTACGGGAAAAG-3′: positions 854–835) and the FAM-labeled probe (5′-FAM-CGTCCGGGATACGAGCGCCCCTT-TAMRA-3′: positions 788–810); *gag*: the forward primer (HTLV-gag-F5 5′-ACCCTTCCTGGGCCTCTATC-3′: positions 1,602–1,621), the reverse primer (HTLV-gag-R5 5′-TCTGGCAGCCCATTGTCAAG-3′: positions 1,695–1,676) and the FAM-labeled probe (HTLV-gag-P5 5′-FAM-ACCACGCCTTCGTAGAACGCCTCAAC-TAMRA-3′: positions 1,644–1,669); *pX*: the forward primer (HTLV-pX2-S 5′-CGGATACCCAGTCTACGTGTT-3′: positions 7,359–7,379), the reverse primer (HTLV-pX2-AS 5′-CAGTAGGGCGTGACGATGTA-3′: positions 7,458–7,439) and the FAM-labeled probe (HTLV-pX2-Probe 5′-FAM-CTGTGTACAAGGCGACTGGTGCC-TAMRA-3′: positions 7,386–7,408).[18,26] A coding region for albumin (*Alb*) was used to measure the copy number of human genome. The primers and the probe for the *Alb* were as follows: The forward primer (Alb-S2 5′-TGTCATCTCTTGTGGGCTGT-3′), the reverse primer (Alb-AS2 5′-GGTTCTCTTTCACTGACATCTGC-3′) and the FAM-labeled probe (Alb-probe 5′-FAM-CCTGTCATGCCCACACAAATCTCTCC-TAMRA-3′). A plasmid containing PCR products for HTLV-1 5′LTR-*gag*, *gag*, *pX* regions and *Alb* was constructed using pGEM T-Easy Vector (Promega Corporation, Madison, WI) and was used as a control template for real-time PCR. PVLs of each region of HTLV-1 provirus were measured in a duplicate manner and were shown as copies per 100 PBMCs.

### Detection of provirus with deletion of HTLV-1 internal sequence by long PCR

To detect the provirus with large deletion of HTLV-1 internal sequence, long PCR, which amplifies provirus maintaining both 5′ and 3′ LTR, was performed as described previously.[19] The primers were as follows: 5′LTR (HTLV-0647F 5′-GTTCCACCCCTTTCCCTTTCATTCACGACTGACTGC-3′: positions 647–682) and 3′LTR (HTLV-8345R 5′-GGCTCTAAGCCCCCGGGGGATATTTGGGGCTCATGG-3′: positions

8,345–8,310).[26] Long PCR was performed using LA Taq Hot start version (Takara Bio, Shiga, Japan). Genomic DNA containing 200 copies of HTLV-1 provirus for the *pX* region was used for this assay. To ensure that the same amount of provirus was used in each reaction, PCR for the *pX* region was performed as an internal control. Primers for this PCR were as follows: the forward primer (HTLV-7396F 5′-GGCGACTGGTGCCC-CATCTCTGGGGGACTATGTTCG-3′: positions 7,396–7,431) and the reverse primer described above (HTLV-8345R). The PCR products were electrophoresed on 0.8% agarose gel and visualized by ethidium bromide staining.

### Detection of provirus with deletion of 5′LTR and its flanking internal sequence by inverse long PCR

As described in results, both *gag* PVL/*pX* PVL ratio and 5′LTR-*gag* PVL/*pX* PVL ratio were low at less than 0.5 in two carriers (C20 and 21) and they were suspected of having provirus with deletion of 5′LTR and its flanking internal sequence. Inverse long PCR (IL-PCR) was used to amplify the genomic DNA adjacent to the 3′LTR of HTLV-1 provirus according to the method described previously with slight modifications.[15] In brief, the genomic DNA was digested with *Kpn* I, *Hind* III, *Sal* I or *Spe* I, and then self-ligated by T4 ligase following digestion with *Mlu* I. Amplification of the resultant DNA was performed using the LA Taq Hot start version. The primers used in this analysis were as follows; a forward primer in the U5 region of the LTR (5′-TGCCTGACCCTGCTTGCTCAACTCTACGTCTTTG-3′: positions 8,856–8,889) and a reverse primer, HTLV-7002R (5′-AGTATTTGAAAAGGAAGGAAGAGGAGAAGGCA-3′: positions 7,002–6,971). Subcloning of the amplified fragments of IL-PCR were subjected to sequencing assay according to the protocol of the Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) using ABI Prism 310 DNA Sequencer (Applied Biosystems) and the human genomic sequence downstream of the HTLV-1 provirus was obtained. The human genomic sequence upstream of the provirus was assumed based on this information by BLAT search (http://genome.ucsc.edu/cgi-bin/hgBlat).[31] The primers for human genomic sequence upstream of the provirus were designed and long PCR was performed using a forward primer (5′-GTGATC-CATGGTGTTTGTCCACCTGAAAGC-3′) and a reverse primer HTLV-7002R in C20, and a forward primer (5′-TCCAAGTGGGATGTCACGGCCACTTCTC-3′) and a reverse primer HTLV-7002R in C21. To determine the upstream junction sequence between host genome and provirus, the PCR products were subjected to direct sequencing using the Big Dye Terminator v1.1 Cycle Sequencing Kit.

### Statistical Analysis

Mann-Whitney's U test was used to compare *pX* PVLs, *gag* PVL/*pX* PVL or 5′LTR-*gag*/*pX* PVL ratios among the groups of asymptomatic HTLV-1 carriers with different infection routes. Spearman's correlation coefficient by rank was used to determine the relationship between *pX* PVL and *gag* PVL/*pX* PVL or 5′LTR-*gag* PVL/*pX* PVL ratio.

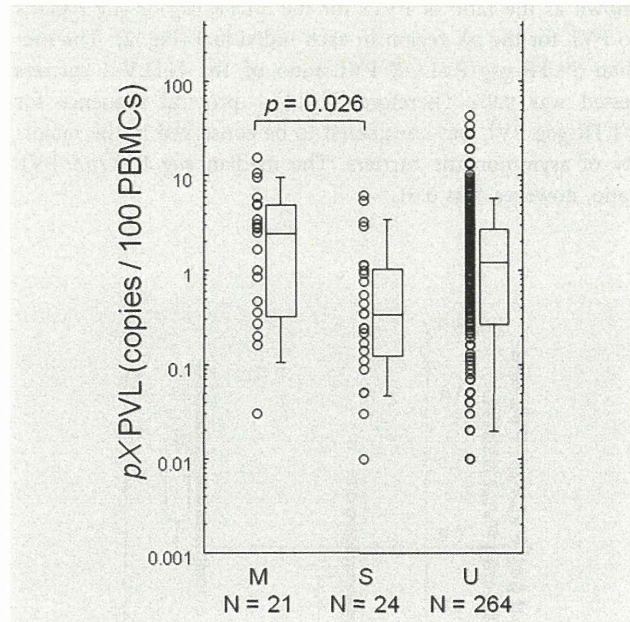


**Figure 1.** *PX* PVLs in HTLV-1 carriers with different infection routes M: Carriers with infection from mothers; S: Carriers with infection from spouses; U: Carriers with undetermined infection routes.

## Results

### pX PVLs in HTLV-1 carriers with different infectious routes

PVLs for the 5′LTR-*gag*, *gag* and *pX* regions in each individual were measured in 309 asymptomatic HTLV-1 carriers. Because the *pX* region has been reported to be conserved in the HTLV-1 provirus, *pX* PVL was considered to represent total PVLs.[23,25] As shown in Figure 1, median *pX* PVL (2.49 copies/100 PBMCs) in 21 asymptomatic carriers, who were infected by their mothers, was significantly higher than that (0.34 copies/100 PBMCs) in 24 carriers who were infected by their spouses ($p = 0.026$). Median *pX* PVL in 264 asymptomatic carriers, whose infection routes were undetermined, was between these values (1.24 copies/100 PBMCs).

### PVLs for 3 different proviral regions (5′LTR-gag, gag and pX) of HTLV-1

To determine whether PVLs for three different proviral regions (5′LTR-*gag*, *gag* and *pX*) of HTLV-1 were equal in asymptomatic carriers, PVLs for the 5′LTR-*gag* and *gag* regions were measured and compared to PVLs for the *pX* region. Because 100 ng of genomic DNA, which is derived approximately 15,000 PBMCs, was used for the template for real time-PCR, 148 carriers with *pX* PVL, which was less than 1 copy/100 PBMCs, were not provided for further analysis to avoid unstable result due to the small number of proviral copies in each reaction. The results of our study were

shown as the ratio of PVLs for the 5′LTR-*gag* or *gag* regions to PVL for the *pX* region in each individual (Fig. 2). The median 5′LTR-*gag* PVL/*pX* PVL ratio of 161 HTLV-1 carriers tested was 0.97. Therefore, HTLV-1 proviral sequence for 5′LTR-*gag* PVL was considered to be conserved in the majority of asymptomatic carriers. The median *gag* PVL/*pX* PVL ratio, however, was 0.61.

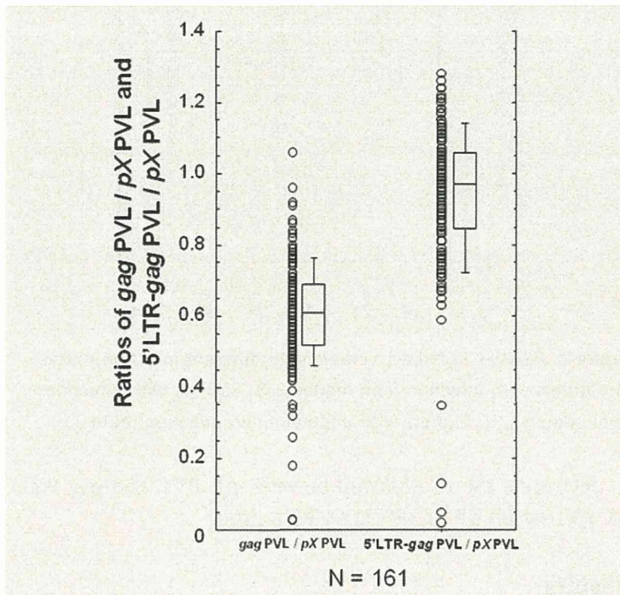


**Figure 2.** The ratios of PVLs for the 5′LTR-*gag* or *gag* regions to PVL for the *pX* region in 161 asymptomatic HTLV-1 carriers, whose *pX* PVLs were equal to or greater than 1 copy/100 PBMCs.

## Detection of provirus with deletion of HTLV-1 internal sequence by long PCR

To determine whether the provirus with deletion of HTLV-1 internal sequence accounted for low *gag* PVL/*pX* PVL ratio, long PCR was performed. For this analysis, we chose 26 carriers with low *gag* PVL/*pX* PVL ratios of less than 0.5; however, adequate DNA sample for long PCR was available in only 17 of the 26 subjects. All subjects except C1 showed a band of 7.7 kb, which was considered to be derived from complete provirus, and some additional smaller bands suggesting defective provirus (Fig. 3*a*). C1 showed only a dense band of 4.5 kb. C1 was analyzed in our previous study and a large deficiency (3.2 kb, positions 1,203–4,368) of internal sequence was shown.[19] Additional four carriers (C3, 4, 11 and 13) showed dense bands equal to or stronger than the band for complete provirus (arrows in Fig. 3*a*). Cloning and DNA sequencing of these dense bands showed large deficiencies of internal sequences (4.9 kb, positions 1,368–6,286 in C3; 0.9 kb, positions 1,413–2,284 in C4; 4.8 kb, positions 1,009–5,763 in C11 and 4.8 kb, positions 1,133–5,974 in C13).

Four carriers (C18–21) had low 5′LTR-*gag* PVL/*pX* PVL ratios of less than 0.5. Long PCR of C18 and 19 showed dense bands of 7.7 kb, which were considered to be derived from complete provirus, and some additional smaller bands (Fig. 3*b*). Polymorphism of proviral DNA sequence of the sites for primers and/or probe for 5′LTR-*gag* PVL was suspected in these two cases, and cloning and DNA sequencing of the PCR products were performed. The polymorphisms of DNA sequence for the annealing site of the forward primer (708 G > A and 709 C > G in C18; 712 C > T in C19) were consistently found, and these polymorphisms were

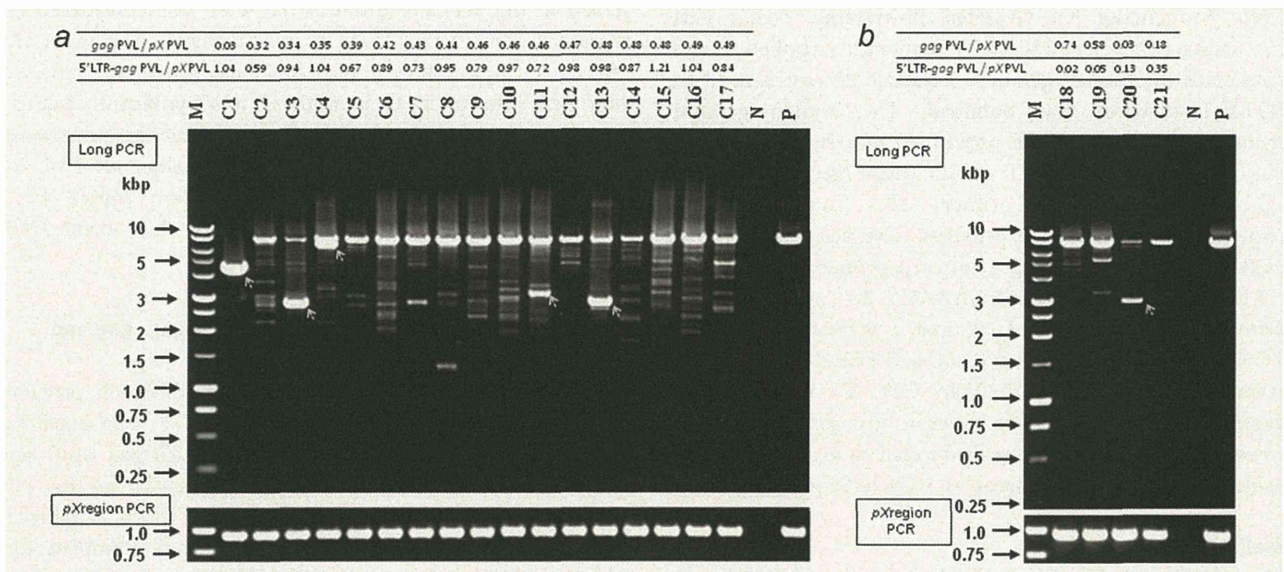


**Figure 3.** Detection of defective provirus by long PCR. (*a*) Asymptomatic HTLV-1 carriers with low *gag* PVL/*pX* PVL ratios less than 0.5. (*b*) Asymptomatic HTLV-1 carriers with low 5′LTR-*gag* PVL/*pX* PVL ratios less than 0.5. Arrows indicate PCR products for HTLV-1 provirus lacking large internal sequence. M: Molecular weight marker; N: HTLV-1-negative subject; P: HTLV-1-positive cell line, ED-40515(-).

considered to account for the decreased efficacy of real time-PCR for 5′LTR-*gag* PVL.

### Detection of provirus with deletion of 5′LTR and its flanking internal sequence by IL-PCR

Both *gag* PVL/*pX* PVL ratio and 5′LTR-*gag* PVL/*pX* PVL ratio were low at less than 0.5 in the additional two carriers (C20 and 21). Long PCR showed a weak band of 7.7 kb for complete provirus and a stronger band of 2.9 kb in C20 (Fig. 3*b*). In the case of C21, only a weak band for complete band was observed (Fig 3*b*). These data suggested defective provirus, which had not been detected by long PCR, existed in C20 and C21. Because these proviruses were suspected of lacking 5′LTR and its flanking internal sequence, we attempted to identify them by IL-PCR. First, the genomic DNA of C20 and C21 were digested with *Kpn* I, *Hind* III, *Sal* I or *Spe* I, and resultant DNA was provided for IL-PCR as a template. In C20, approximately 1.1 kb of PCR product was obtained in digestion with *Kpn* I alone (Fig. 4*a-1*). No IL-PCR product was obtained using other restriction enzymes (data not shown). When this PCR product was digested with *Kpn* I, two major bands appeared, as expected (Fig. 4*a-1*). Cloning and sequencing revealed that this product consisted of HTLV-1 provirus (*Kpn* I site at position: 6,141 to the end of 3′LTR) and its flanking genomic DNA of human chromosome 2 (2q13). Based on the information obtained, a forward primer to anneal the upstream human genome adjuncted to the provirus was prepared and clone-specific PCR was performed. Cloning and sequencing of this clone-specific PCR product revealed that it lacked 5′LTR and its internal flanking sequence (until position 5,999; Fig. 4*a-2*). In the case of C21, IL-PCR product was obtained in digestion with *Hind* III alone. Following the same procedure as in C20, it was revealed that a provirus integrated in human chromosome 18 (18p11.32), and that it lacked 5′LTR and its internal flanking sequence (until position 4,976) (Figs. 4*b-1* and 4*b-2*).

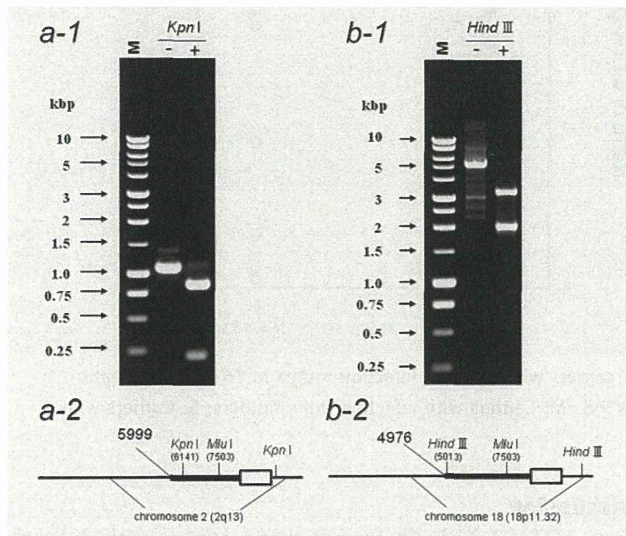


**Figure 4.** Detection of provirus with deletion of 5′LTR and its internal flanking sequence by IL-PCR. (*a-1*) Long PCR products from an asymptomatic HTLV-1 carrier, C20, with or without *Kpn* I digestion. (*a-2*) Scheme of the structure of defective provirus in C20. (*b-1*) Long PCR products from an asymptomatic HTLV-1 carrier, C21, with or without Hind III digestion. (*b-2*) Scheme of the structure of defective provirus in C21.

### Relationship between pX PVL and gag PVL/pX PVL or 5′LTR-gag/pX PVL ratios

To determine whether the HTLV-1 PVLs correlated with the number of provirus with deficiency and/or polymorphism of the *gag* or 5′LTR-*gag* regions, the relationship between *pX* PVL and *gag* PVL/*pX* PVL or 5′LTR-*gag*/*pX* PVL ratios was analyzed. As shown in Figure 5*a*, there was a negative

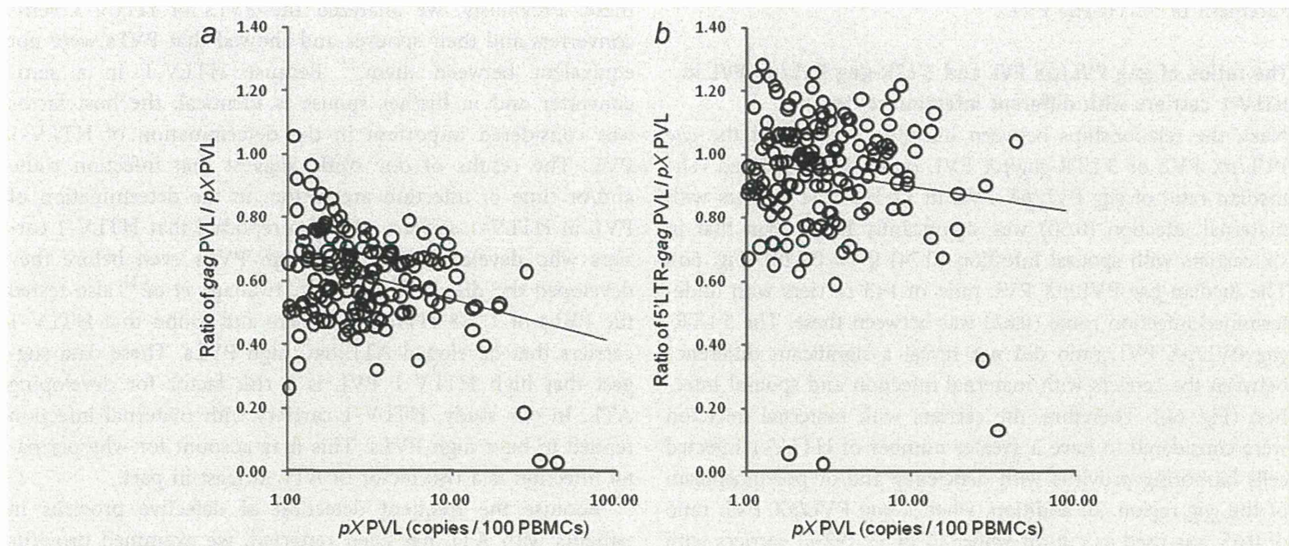


**Figure 5.** Relations of *pX* PVL and *gag* PVL/*pX* PVL or 5′LTR-*gag* PVL/*pX* PVL ratios in 161 asymptomatic carriers. (*a*) Relation of *pX* PVL and *gag* PVL/*pX* PVL. (*b*) Relation of *pX* PVL and 5′LTR-*gag* PVL/*pX* PVL.

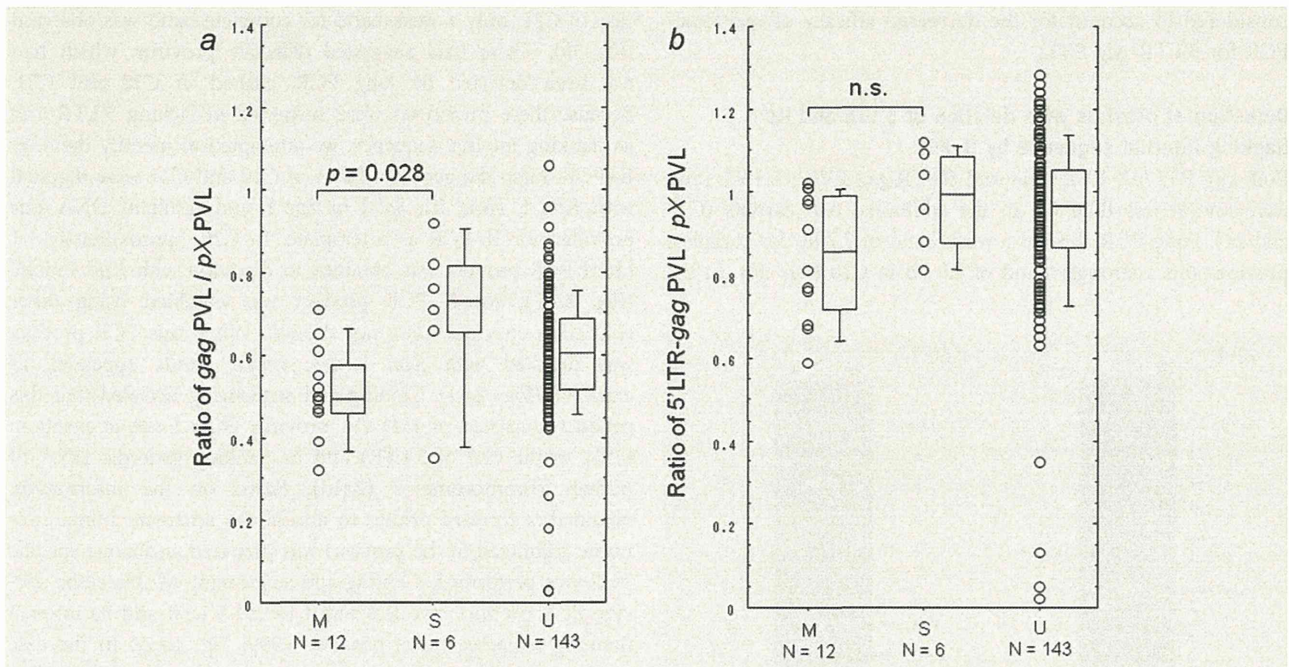


**Figure 6.** The ratios of *gag* PVL/*pX* PVL or 5′LTR-*gag* PVL/*pX* PVL in HTLV-1 carriers with different infection routes in 161 asymptomatic carriers. (*a*) The ratio of *gag* PVL/*pX* PVL. (*b*) The ratio of 5′LTR-*gag* PVL/*pX* PVL. M: Carriers with infection from mothers; S: Carriers with infection from spouses; U: Carriers with undetermined infection routes.



correlation between *pX* PVL and the *gag* PVL/*pX* PVL ratio ($r = -0.46$, $p = 0.02$). Therefore, HTLV-1 infected cells harboring provirus with deficiency and/or polymorphism of the *gag* region were considered to be more prevalent in asymptomatic carriers with high PVL. In the case of 5′LTR-*gag*/*pX* PVL ratio, the trend was not obvious (Fig. 5*b*) ($r = -0.20$, $p = 0.94$). However, variability of the 5′LTR-*gag*/*pX* PVL ratio was greater than that of *gag* PVL/*pX* PVL ratio. This may have been the result of technical inadequacies in the measurement of 5′LTR-*gag* PVL.

### The ratios of gag PVL/pX PVL and 5′LTR-gag PVL/pX PVL in HTLV-1 carriers with different infection routes

Next, the relationships between infection routes and the *gag* PVL/*pX* PVL or 5′LTR-*gag*/*pX* PVL ratios were analyzed. The median ratio of *gag* PVL/*pX* PVL in 12 HTLV-1 carriers with maternal infection (0.50) was significantly lower than that in six carriers with spousal infection (0.74) ($p = 0.028$) (Fig. 6*a*). The median *gag* PVL/*pX* PVL ratio of 143 carriers with undetermined infection route (0.62) was between these. The 5′LTR-*gag* PVL/*pX* PVL ratio did not reveal a significant difference between the carriers with maternal infection and spousal infection (Fig. 6*b*). Therefore, the carriers with maternal infection were considered to have a greater number of HTLV-1 infected cells harboring provirus with deficiency and/or polymorphism of the *gag* region. In addition, when a *gag* PVL/*pX* PVL ratio of 0.65 was used as cut-off value, 11 of 12 (92%) carriers with maternal infection, against only one of six (17%) carriers with spousal infection, showed lower values.

## Discussion

First, HTLV-1 PVLs in asymptomatic carriers with different infection routes were analyzed. *PX* PVL in 21 asymptomatic carriers with maternal infection was significantly higher than that in 24 carriers with spousal infection. These results agreed with data reported by Roucoux *et al.*[32] showing that PVLs in index HTLV-1 positive carriers were higher than those of their newly infected partners. Asymptomatic carriers whose infection routes were undetermined showed values between these. Previously, we analyzed the PVLs of HTLV-1 seroconverters and their spouses and showed that PVLs were not equivalent between them.[22] Because HTLV-1 in a seroconverter and in his/her spouse is identical, the host factor was considered important in the determination of HTLV-1 PVL. The results of our study suggest that infection route and/or time of infection are factors in the determination of PVL in HTLV-1 carriers. We also reported that HTLV-1 carriers who developed ATL had high PVLs even before they developed the disease.[20] Recently, Iwanaga *et al.*[21] also tested the PVLs of 1,218 HTLV-1 carriers and found that HTLV-1 carriers that developed ATL had high PVLs. These data suggest that high HTLV-1 PVL is a risk factor for developing ATL. In our study, HTLV-1 carriers with maternal infection tended to have high PVLs. This may account for why perinatal infection is a risk factor of ATL at least in part.

Because the frequent detection of defective provirus in patients with ATL has been reported, we examined provirus with deficiencies and/or polymorphism of proviral sequence in asymptomatic HTLV-1 carriers. The *pX* region has been

reported to be conserved in HTLV-1 provirus, and PCR for this region was used to measure total PVL.[23,25] Ohshima *et al.*[25] reported that variation of DNA sequence is frequently detected in the *gag* region of HTLV-1 provirus in patients with ATL. Kamihira *et al.*[24] also reported that most of deficient provirus in patients with ATL lacked part of the *gag* region in the proviral regions of HTLV-1 tested. HTLV-1 provirus with deletion of the 5′LTR, and its flanking internal sequences was also found in patients with ATL.[26] In our study, therefore, we tried to find provirus with deficiencies and/or polymorphism of DNA sequence in the asymptomatic carriers by measuring PVLs for the *gag* and 5′LTR-*gag* regions as ratios to *pX* region PVLs. As a result, median 5′LTR-*gag* PVL/*pX* PVL and *gag* PVL/*pX* PVL ratios of 161 HTLV-1 carriers with relatively high *pX* PVL (equal to or greater than one copy per 100 PBMCs) were 0.97 and 0.61, respectively. Our interpretation of this result was that many HTLV-1 infected cells in asymptomatic carriers harbor provirus with deficiency and/or polymorphism of DNA sequences for the sites of primers and/or probe for *gag* real time-PCR.

Long PCR analysis was performed on 17 carriers with low *gag* PVL/*pX* PVL ratios. Five of 17 carriers (29%) were shown to have the provirus with large deletions of internal DNA sequence including the *gag* region. The clonal expansion of HTLV-1 infected cells harboring defective provirus in these five carriers was most likely. In fact, clonal expansion of HTLV-1 infected cells in C1 was already shown in our previous study.[19] The reason for the low *gag* PVL/*pX* PVL ratios in the other 12 carriers was not clear. Contribution of the sum total of HTLV-1 infected cells with defective provirus, which did not reveal dense bands, was possible. Alternatively, polymorphism of the proviral DNA sequence for the *gag* region may have decreased the efficiency of real time-PCR for *gag* PVL. However, cloning and DNA sequencing of the sites for primers and probes for real time-PCR for *gag* PVL in these carriers did not show consistent polymorphism of the proviral DNA (data not shown). This may be because there is high diversity of proviral DNA sequence in the *gag* region of HTLV-1 and it was not possible to prepare cloning primers to work for all of them.

The other two (C20 and 21) showed low ratios not only of 5′LTR-*gag* PVL/*pX* PVL but also of *gag* PVL/*pX* PVL. Our previous study showed that they had high PVLs and clonal expansion of HTLV-1 infected cells with defective provirus.[19] We could not identify the type of defective provirus in the previous study. In our study, however, we found provirus lacking 5′LTR and its internal flanking region existed in these carriers.

In our study, the provirus with deficiency and/or polymorphism of the *gag* region was commonly found in asymptomatic HTLV-1 carriers. Few carriers had provirus lacking 5′LTR and its flanking sequence. Carriers with provirus with deficiency and/or polymorphism of the *gag* region were found frequently among asymptomatic carriers with high PVLs. These infected cells may not express certain HTLV-1 proteins. This change may make it possible for the HTLV-1 infected cells to avoid attack by cytotoxic T-lymphocytes.[33] Therefore, there is a possibility that provirus with deficiency and/or polymorphism of HTLV-1 provirus contributes to the survival of HTLV-1 infected cells. Indeed, our previous study showed that C1, 20 and 21 had clonal expansion of HTLV-1 infected cells.[19]

Low *gag* PVL/*pX* PVL ratio was found to be associated with maternal infection. The reason carriers with maternal infection have a greater number of HTLV-1 infected cells harboring provirus with deficiency and/or polymorphism of the *gag* region was not clear in our study. The replication of HTLV-1 infected cells in long-term infected carriers may account for this. Alternatively, a low level of new cell to cell infection *in vivo* can contribute to the creation of deficiency and/or polymorphism in proviral genome.

Maternal infection has been considered to be a risk factor for the development of ATL in asymptomatic carriers. However, there has been no method to identify infection route in the absence of information on family HTLV-1 status. The results of our study suggest the possibility that *gag* PVL/*pX* PVL ratio can be used as a tool to differentiate the infection routes of asymptomatic HTLV-1 carriers. Due to the fact that only a small number of HTLV-1 carriers with known infectious routes were analyzed in our study, further study with a larger number of subjects is necessary.

A major limitation of our study is that the subjects were elderly individuals, whose median age was 67 years old. The average age at onset of ATL was reported as 60 years.[34] Therefore, it is not clear whether the same result would be obtained from an analysis of younger HTLV-1 asymptomatic carriers. In addition, carriers with low *pX* PVL (less than 1 copy/100 PBMCs) were not provided for the analysis of deficiency and/or polymorphism of HTLV-1 proviral sequence because of technical limitations. Further analysis of carriers with low PVLs using improved methodology is necessary.

In conclusion, our study showed that *pX* PVL in carriers with maternal infection was significantly higher than that in carriers with spousal infection. Low *gag* PVL/*pX* PVL ratio reflecting deficiency and/or polymorphism in proviral genome was associated with high PVLs and maternal infection. These data suggest that development of ATL in carriers with maternal infection may be due in part to high PVL, which can be related to provirus with deficiency and/or polymorphism in proviral genome. In addition, *gag* PVL/*pX* PVL ratio has potential for use as a tool to differentiate infection routes of asymptomatic HTLV-1 carriers. Further study is necessary to clarify the mechanism of deficiency and/or polymorphism in HTLV-1 proviral genome and its implications in ATL development.

## Acknowledgements

The authors thank Ms. Y. Kaseda and Ms. N. Kanemaru (Miyazaki University) for their technical assistance and Dr. M. Maeda (Kyoto University) for the gift of the HTLV-1 infected cell line, ED-40515(-).

## References


1. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 1977;50:481–92.
2. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci USA* 1982;79: 2031–5.
3. Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Matsumoto M, Tara M. HTLV-I associated myelopathy, a new clinical entity. *Lancet* 1986;1: 1031–2.
4. Gessain A, Barin F, Vernant JC, Gout O, Maurs L, Calender A, de The G. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* 1985;2: 407–10.
5. Hino S, Yamaguchi K, Katamine S, Sugiyama H, Amagasaki T, Kinoshita K, Yoshida Y, Doi H, Tsuji Y, Miyamoto T. Mother-to-child transmission of human T-cell leukemia virus type 1. *Jpn J Cancer Res (Gann)* 1985;76:474–80.
6. Kajiyama W, Kashiwagi S, Ikematsu H, Hayashi J, Nomura H, Okochi K. Intrafamilial transmission of adult T cell leukemia virus. *J Infect Dis* 1986;154: 851–7.
7. Okochi K, Sato H, Hinuma Y. A retrospective study on transmission of adult T cell leukemia virus by blood transfusion: seroconversion in recipients. *Vox Sang* 1984;46:245–53.
8. Arisawa K, Soda M, Endo S, Kurokawa K, Katamine S, Shimokawa I, Koba T, Takahashi T, Saito H, Doi H, Shirahama S. Evaluation of adult T-cell leukemia/ lymphoma incidence and its impact on non-Hodgkin lymphoma incidence in southwestern Japan. *Int J Cancer* 2000;85: 319–24.
9. Yamaguchi K, Watanabe T. Human T lymphotropic virus type-I and adult T-cell leukemia in Japan. *Int J Hematol* 2002; 76(Suppl 2):240–5.
10. Murphy EL, Hanchard B, Figueroa JP, Gibbs WN, Lofters WS, Campbell M, Goedert JJ, Blattner WA. Modelling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type I. *Int J Cancer* 1989;43:250–3.
11. Bartholomew C, Jack N, Edwards J, Charles W, Corbin D, Cleghorn FR, Blattner WA. HTLV-I serostatus of mothers of patients with adult T-cell leukemia and HTLV-I-associated myelopathy/tropical spastic paraparesis. *J Hum Virol* 1998;1: 302–5.
12. Wilks R, Hanchard B, Morgan O, Williams E, Cranston B, Smith ML, Rodgers-Johnson P, Manns A. Patterns of HTLV-I infection among family members of patients with adult T-cell leukemia/ lymphoma and HTLV-I associated myelopathy/tropical spastic paraparesis. *Int J Cancer* 1996; 65:272–3.
13. Seiki M, Eddy R, Shows TB, Yoshida M. Nonspecific integration of the HTLV provirus genome into adult T-cell leukaemia cells. *Nature* 1984; 309:640–2.
14. Wattel E, Vartanian JP, Pannetier C, Wain-Hobson S. Clonal expansion of human T-cell leukemia virus type I-infected cells in asymptomatic and symptomatic carriers without malignancy. *J Virol* 1995;69: 2863–8.
15. Etoh K, Tamiya S, Yamaguchi K, Okayama A, Tsubouchi H, Ideta T, Mueller N, Takatsuki K, Matsuoka M. Persistent clonal proliferation of human T-lymphotropic virus type I-infected cells in vivo. *Cancer Res* 1997;57:4862–7.
16. Cavrois M, Leclercq I, Gout O, Gessain A, Wain-Hobson S, Wattel E. Persistent oligoclonal expansion of human T-cell leukemia virus type 1-infected circulating cells in patients with Tropical spastic paraparesis/HTLV-1 associated myelopathy. *Oncogene* 1998;17:77–82.
17. Gillet NA, Malani N, Melamed A, Gormley N, Carter R, Bentley D, Berry C, Bushman FD, Taylor GP, Bangham CR. The host genomic environment of the provirus determines the abundance of HTLV-1-infected T cell clones. *Blood* 2011;117: 3113–22.
18. Tanaka G, Okayama A, Watanabe T, Aizawa S, Stuver S, Mueller N, Hsieh CC, Tsubouchi H. The clonal expansion of human T lymphotropic virus type 1-infected T cells: a comparison between seroconverters and long-term carriers. *J Infect Dis* 2005;191: 1140–7.
19. Takenouchi H, Umeki K, Sasaki D, Yamamoto I, Nomura H, Takajo I, Ueno S, Umekita K, Kamihira S, Morishita K, Okayama A. Defective human T-lymphotropic virus type 1 provirus in asymptomatic carriers. *Int J Cancer* 2010; 128:1335–43.
20. Okayama A, Stuver S, Matsuoka M, Ishizaki J, Tanaka G, Kubuki Y, Mueller N, Hsieh CC, Tachibana N, Tsubouchi H. Role of HTLV-1 proviral DNA load and clonality in the development of adult T-cell leukemia/lymphoma in asymptomatic carriers. *Int J Cancer* 2004; 110:621–5.
21. Iwanaga M, Watanabe T, Utsunomiya A, Okayama A, Uchimaru K, Koh KR, Ogata M, Kikuchi H, Sagara Y, Uozumi K, Mochizuki M, Tsukasaki K, et al. Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan. *Blood* 2010;116:1211–9.
22. Iga M, Okayama A, Stuver S, Matsuoka M, Mueller N, Aoki M, Mitsuya H, Tachibana N, Tsubouchi H. Genetic evidence of transmission of human T cell lymphotropic virus type 1 between spouses. *J Infect Dis* 2002;185:691–5.
23. Tamiya S, Matsuoka M, Etoh K, Watanabe T, Kamihira S, Yamaguchi K, Takatsuki K. Two types of defective human T-lymphotropic virus type I provirus in adult T-cell leukemia. *Blood* 1996;88: 3065–73.
24. Kamihira S, Sugahara K, Tsuruda K, Minami S, Uemura A, Akamatsu N, Nagai H, Murata K, Hasegawa H, Hirakata Y, Takasaki Y, Tsukasaki K, et al. Proviral status of HTLV-1 integrated into the host genomic DNA of adult T-cell leukemia cells. *Clin Lab Haematol* 2005;27: 235–41.
25. Ohshima K, Kikuchi M, Masuda Y, Kobari S, Sumiyoshi Y, Eguchi F, Mohtai H, Yoshida T, Takeshita M, Kimura N. Defective provirus form of human T-cell leukemia virus type I in adult T-cell leukemia/lymphoma: clinicopathological features. *Cancer Res* 1991;51:4639–42.
26. Miyazaki M, Yasunaga J, Taniguchi Y, Tamiya S, Nakahata T, Matsuoka M. Preferential selection of human T-cell leukemia virus type 1 provirus lacking the 5' long terminal repeat during oncogenesis. *J Virol* 2007;81: 5714–23.
27. Korber B, Okayama A, Donnelly R, Tachibana N, Essex M. Polymerase chain reaction analysis of defective human T-cell leukemia virus type I proviral genomes in leukemic cells of patients with adult T-cell leukemia. *J Virol* 1991;65: 5471–6.
28. Yoshida M. Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu Rev Immunol* 2001;19: 475–96.
29. Mueller N, Okayama A, Stuver S, Tachibana N. Findings from the Miyazaki

Cohort Study. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;13(Suppl 1): S2–7.

30. Seiki M, Hattori S, Hirayama Y, Yoshida M. Human adult T-cell leukemia virus: complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. *Proc Natl Acad Sci USA* 1983;80: 3618–22.
31. Kent WJ. BLAT—the BLAST-like alignment tool. *Genome Res* 2002;12: 656–64.
32. Roucoux DF, Wang B, Smith D, Nass CC, Smith J, Hutching ST, Newman B, Lee TH, Chafets DM, Murphy EL. A prospective study of sexual transmission of human T lymphotropic virus (HTLV)-I and HTLV-II. *J Infect Dis* 2005;191:1490–7.
33. Kannagi M. Immunologic control of human T-cell leukemia virus type I and adult T-cell leukemia. *Int J Hematol* 2007; 86:113–7.
34. Matsuoka M, Jeang KT. Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat Rev Cancer* 2007;7:270–80.

特集／ストップ ザ 性感染症

性感染症－診断・治療

# HTLV-1 感染

岡 山 昭 彦

## Ⅰ．ヒトTリンパ向性ウイルス 1型（HTLV-1）

HTLV-1は1981年，米国のGallo，本邦の日沼らによって発見されたC型レトロウイルスである[1]。HTLV-1によって引き起こされる主な疾患として成人T細胞性白血病（ATL）やHTLV-1関連脊髄症（HAM），HTLV-1関連ぶどう膜炎（HU）がある[1]。感染の主なターゲットはCD4陽性Tリンパ球であるが，フリーのウイルス粒子による感染効率は極めて低く，感染細胞と非感染細胞が直接接触することにより新規感染が成立する。このような機会は自然界では母子間，配偶者間などの男女間等に限られている。細胞－細胞間接触によりHTLV-1に感染したリンパ球では逆転写酵素により相補的DNAが形成される。この相補的DNAはリンパ球のゲノムDNAに組み込まれ，プロウイルスとなる。ヒトからヒトに感染するウイルスであるにもかかわらず，HTLV-1の感染力が非常に弱いために浸淫地域は限られている。世界におけるHTLV-1感染者は約2,000万人という推測がある。主な浸淫地域は日本，カリブ海沿岸諸国，南米，南－中央アフリカ，メラネシア，パプアニューギニアなどである。これらのサブグループはおおよそ，そのウイルスキャリアの居住地と一致しており，民族やヒトの移動といった人類学的背景を反映したものと考えられる。本邦においては1980年代の疫学研究により全国の感染者は120万人と推測され，九州，沖縄を中心とした西南日本に高頻度であることが示された。この調査の時点では感染者（キャリア）数はその後減少すると予想されていたが，約20年後の2007年に行われた疫学調査による全国の推定キャリア数は108万人であり，当初予想されたよりもあまり減少していないことが判明した[2]。さらに地域的な広がりとして西南日本におけるキャリア数は減少傾向であるのに対して，関東，中部圏などの都市部においては相対的に増加傾向にあることも示された。

宮崎大学医学部内科学講座免疫感染病態学分野

## Ⅱ．無症候性キャリアと疾患

HTLV-1の新規感染では，感染初期に発熱やリンパ節腫脹などHIV初感染で見られるような症状は報告されておらず，抗体陽性の無症候性キャリアとなる。抗体陽転直後の末梢血液単核細胞中の感染細胞の割合は（1個の細胞にプロウイルス1コピー感染していると仮定すると）0.1%以下と少ないものから10%以上まで個体により1,000倍以上の大きな差がある。このような状態の感染者に症状はないが，末梢血液スメアにおいては異型な核を有するリンパ球がみられることがある。キャリアの95%は生涯無症候性であるが，一部のキャリアからATLやHAM，HU等の発症がみられる。

### 1. ATL

ATLは高月らによって1977年に報告された疾患であり，HTLV-1感染Tリンパ球がモノクローナルに増殖し，腫瘍化したものである。キャリアからのATL生涯発症率は約2～5%と推測されており，ATL患者のほとんどは母児間感染により乳児期以前に感染し，通常50年以上の潜伏期間をへて発症するものと推測されている。最近の疫学調査では平均発症年齢は67歳とされている。キャリアが女性に多いのに対して，ATL患者は男性にやや多い。日本全体で年間約1,000例の患者死亡があるとされている。ATLの主な症状，所見は，発熱，倦怠感，リンパ節腫大，皮疹，肝脾腫などであり，末梢

血液に特徴的な花弁状の核を有する白血病細胞を認める。白血病細胞にはHTLV-1プロウイルスがモノクローナルに組み込まれており，これをサザンブロット法により検出することによって確定診断することができる。主な合併症として高カルシウム血症や日和見感染症がある。ATLは抗がん剤多剤併用化学療法に抵抗性であり，易感染性のためもあって予後不良な疾患であるが，骨髄移植の併用やCCR4に対する抗体療法など新たなATLの治療法の開発が進められている。

**2.　HAM**

1986年に納らがHTLV-1キャリアの一部が慢性進行性の痙性脊髄麻痺を示すことを見出し，新しい疾患単位，HAMとして報告した。ほぼ同じころカリブ海沿岸で同様の症状とHTLV-1感染との関連が示され，熱帯性痙性麻痺 tropical spastic paraparesisとして報告されていたため，HAM/TSPと呼ばれることもある。本邦での正確な患者数は把握されていないがキャリア約1,000人にひとり程度存在するとされており，ATLと異なって若い年齢層にも発症する。平均発症年齢は45歳であり，男女比も1：2と女性に多い。HTLV-1の感染経路としては母子間，配偶者間，輸血のどの経路からも発症する。輸血による症例は，血液センターにおけるHTLV-1のスクリーニング開始以降見られなくなった。症状としては，慢性進行性の痙性脊髄麻痺が主体で膀胱直腸障害を伴うことが多い。治療としては，ステロイド剤により臨床症状の改善が認められる症例があり，またインターフェロンも有効であることが判明し保険適応にもなっている。

**3.　HU**

1992年に望月らにより提唱された疾患で本邦における頻度はキャリア1,000人に対して1～2人と報告されている。男女比は1：2と女性に多く，家族内発症の報告がみられる。片眼または両眼のブドウ膜炎で，症状は霧視，飛蚊症，視力低下などである。治療としてはステロイドの局所または全身投与が行われよく奏効するが，半数が再発するといわれている。

**4.　そのほかの疾患との関連**

上記の疾患の他にHTLV-1高浸淫地域において関節炎，膠原病，肺病変，皮膚疾患など種々の慢性炎症疾患と本ウイルスとの関連が示唆されている。

## Ⅲ．HTLV-1 感染症の診断

HTLV-1キャリアは抗体陽性であり陰性化することはないと考えられているため，HTLV-1感染のスクリーニングには抗体が測定される。スクリーニング法としては酵素免疫法や粒子凝集法が一般的に用いられるが，偽陽性があるため，陽性の場合はウエスタンブロット法あるいは蛍光抗体法など他の方法による確認が必要になる。抗体陽性であればHTLV-1キャリアと診断される。ウイルスそのものの分離同定は日常の臨床検査では行われず，HTLV-1そのものの検出にはウイルス遺伝子の検出をPCR法により行うが，いまだ研究的な検査であり，保険適応になっていない。

## Ⅳ．HTLV-1の感染経路（表1）

前述したように，ヒトからヒトへのHTLV-1新規感染の成立にはキャリアの感染細胞が非感染者の体内に入り，細胞間接触が起こることが必要である。疫学的にも，感染が基本的には家族内で成立することが強く示唆されている[3)]。

家族内感染が主な感染経路であるため，地域における感染率は隣接する地域，市町村単位であっても大きく異なる。このため周囲との交通の不便な離島や集落単位で陽性率の高い地域がある。このような地域における1980年代の研究では，感染率は年齢が高いほど高く，また特に中年以降において女性が男性よりも高いことが示された（図1）。年齢が高くなるほど感染率が高い理由としては2つ考えられる。ひとつは出生コホート効果とよばれるもので，年齢が高い人たちの陽性率が高いのは，その人々が出生した時代における感染率（おそらく母児感染の頻度）が高かったことを反映したというものである。もうひとつの考え方は後述するように配

**表 1**　HTLV-1感染経路と頻度

| | | |
|---|---|---|
| 1. | 母子感染 | |
| | 母乳による感染 | 20%前後 |
| | それ以外（経産道？） | 2～3% |
| 2. | 配偶者間感染 | 不明であるが男性から女性の頻度が高い |
| 3. | 輸血による感染 | 1986年以降なし |

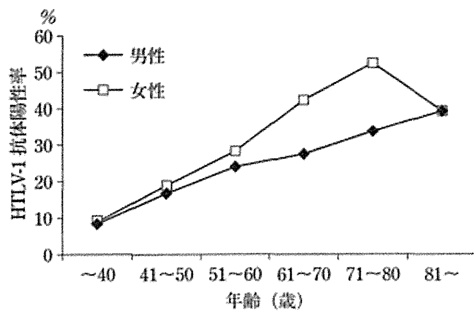


図 1　HTLV-1高浸淫地域における年齢男女別抗体陽性率

偶者間の新規感染のため一定の年齢以降の感染率が高くなるというものである。

### 1. 母子感染

HTLV-1の母子感染については，疫学的，実験的な結果から主な経路は母乳を介したものであることが判明した。ATLは母子間感染により乳児期に感染したキャリアから発症すると考えられているため，母乳を介した母子感染を遮断し，ATLを主とする将来のHTLV-1関連疾患の発症を予防しようとする試みが行われてきた。すなわち抗体が陽性と判明した妊婦のなかで，母乳を与えないことに同意したキャリア母親には出産後母乳分泌抑制剤が投与され児の人工栄養が行われた。この結果，キャリア母親から出生した児の感染率は，6ヵ月以上の母乳栄養で20.5%であるのに対して，人工栄養では2.4%と有意に低下することが示された[4)5)]。

### 2. 性行為に伴う感染

HTLV-1の配偶者間感染については，配偶者間でのHTLV-1陽性の一致率が高く，とくに年齢の高い夫婦ほど配偶者両者ともHTLV-1が陽性となる率が高いことが判明している。またHTLV-1キャリアの陰性の配偶者（たとえば夫が陽性で妻陰性）を長期にフォローすると，陰性であった配偶者の抗体陽転化が一定の割合で生じる（妻が陽性になる）[6)7)]。さらにこのようなケースにおける両者のウイルスの遺伝子配列を比較すると同一であることから，配偶者間感染が証明されている[8)]。感染は女性から男性においても成立しうるが，男性から女性において頻度が高い。このことは疫学的に，HTLV-1抗体陽性率が中年以降において女性のほうが男性よりも高いという事実と合致している。配偶者間感染が性行為に伴って起こることについての直接的な証拠はない。しかし，HTLV-1陽性男性の精液に感染性が証明されていることや[9)]，このウイルスの感染には細胞間接触が必要なことを合わせて考えると，性行為に伴っての感染が最も考えやすい。成人における配偶者感染によってATLが発症したという明らかな報告はないが，完全に否定されたわけではない。また成人における感染においてもHAMやHU等のHTLV-1関連疾患の発症は認められている。母子感染においては母乳哺育遮断という感染防止の方法論が確立されているが，性行為感染においてはこのような感染防止法として確立したものはないのが現状である。

## V. HTLV-1感染症への対策

前述したように，母子感染については母乳を介した感染の遮断により感染率を大幅に減少させることに成功している。しかしながら，母乳感染遮断の介入を行った場合にも2～3%程度の児に感染が成立することが判明しており，母乳遮断のみによる母子感染防止には課題が残る。今後の可能性のひとつとしてワクチンによる感染予防法の開発がある。これが実現すれば，現在全く対策のない配偶者間感染や医療機関における針刺し事故などの対策においても福音となると思われる。HTLV-1感染に対するワクチン開発も基礎研究レベルでは開始されており，今後さらなる発展が期待される。また新たな感染を予防することに加えて，現在国内に108万人いると考えられているHTLV-1キャリアについてはHTLV-1関連疾患発症予防法や治療法の開発が必要である。これまでの研究から，感染細胞数の多いキャリアからHTLV-1関連疾患が発症することが判明しており[10)]，今後キャリアのウイルス量を減らす方法が開発されれば，HTLV-1関連疾患の発症防止に大きく貢献できる可能性がある。

2010年に国において「HTLV-1総合対策」が取りまとめられ，HTLV-1感染予防，相談支援，医療体制の整備，啓発・情報提供，研究開発の推進が重点施策として挙げられており，今後の発展が期待されている。

## まとめ

本ウイルスが発見されて30年が経過し，ウイ