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Original article

Distribution of Two Subgroups of Human T-Lymphotropic Virus Type 1 (HTLV-1) in Endemic Japan

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Abstract: Endemic areas of human T-lymphotropic virus type 1 (HTLV-1) have been reported in Japan as well as tropical Africa, Central and South America and Melanesia. The existence of two subgroups, i.e., the transcontinental and Japanese subgroups, was reported in Japan. In the present study, we provide data on the ratio of the two subgroups in each endemic area and infection foci and examine the distribution of HTLV-1 in Japan and neighboring areas. A 657 bp fragment of env region of HTLV-1 proviral genome was successfully amplified for 183 HTLV-1 positive DNA samples. The subgroup determination was done by RFLP reactions using endonucleases *HpaI* and *HinfI*. The northern part of mainland Kyushu, represented by Hirado and Kumamoto, was monopolized by the Japanese subgroup, while the transcontinental subgroup ranged from 20 to 35% in the Pacific coast areas of Shikoku (Kochi), the Ryukyu Archipelago (Kakeroma and Okinawa) and Taiwan. An interesting finding in the present study is the presence of the transcontinental subgroup in Kochi, suggesting the endemicity of the transcontinental subgroup along the Kuroshio Current.
Key words: Japanese subgroup, transcontinental subgroup, human migration, Kuroshio Current

INTRODUCTION

Human T-lymphotropic virus type 1 (HTLV-1) was first isolated in 1980 [1] and has been identified as a causative agent of adult T cell leukemia (ATL) and HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-1 has three major transmission routes: from mother to infant through breast milk, from male to female through semen, and to blood recipients through the lymphocytes of HTLV-1 carriers. These transmission routes, especially mother-to-child transmission, allow HTLV-1 to pass from generation to generation and localize within family, community and ethnic groups. Thus, the elucidation of the geographical distribution of HTLV-1 has important ethno-epidemiological implications [2].

In view of this unique fact, a large number of phylogeographycal and epidemiological studies have been conducted within and beyond the borders of Japan, and valuable results have been obtained. Firstly, endemic areas were reported in tropical Africa, the Caribbean basin, Central and South America, Papua New Guinea and other islands of Melanesia, as well as Japan [3, 4]. Secondly, there are three major lineages existing worldwide: the Melanesian subtype, the Central African subtype, and the cosmopolitan subtype, ubiquitous in endemic areas around the world [5, 6]. Thirdly, the cosmopolitan subtype is further divided into three major subgroups: A, B, and C, which correspond to the transcontinental subgroup, the Japanese subgroup, and the West African subgroup, respectively [7, 8]. Fourthly, within Japan, endemicity is found in Kyushu and Okinawa, and small infection foci are seen in coastal islands of the Japan Sea and the Pacific side of Shikoku, Kii and Tohoku, while most of Honshu is HTLV-1-free [3]. Furthermore, a few endemic areas have been found in areas neighboring Japan: Nogliki of Sakhalin, Kinmen, Fujian and Taiwan [7, 9–11]. Fifthly, the existence of two different subgroups of

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HTLV-1, i.e., the transcontinental and Japanese subgroups, in Japan and clusters of the former subgroup in Kyushu and the Ryukyu islands were reported [12].

In the present study, we provide data on the ratio of the two subgroups in each endemic area and infection foci within Japan and use that data to elucidate the distribution of HTLV-1 in Japan and neighboring area.

MATERIALS AND METHODS

DNA samples from a total of 197 anonymous HTLV-1 positive donors were obtained from the Joint Study on Predisposing Factors of ATL Development (JSPFAD) and used in the present study. Of the 197 samples, 40 were gathered in Hokkaido (Hokkaido University Hospital), four in Iwate (Iwate Medical University), 30 in Kochi (Kochi Medical School Hospital), 50 in Hirado (Nagasaki University Hospital), 23 in Kumamoto (Kumamoto University Hospital) and 50 in Okinawa (Okinawa Kyodo Hospital).

Furthermore, DNA was extracted from peripheral blood donated by five anonymous HTLV-1 carriers on Ishigaki Island, Japan (Yaeyama County, Ishigaki City, Okinawa Prefecture). The analysis of samples donated by the Yaeyama residents was approved by the ethics committee of the Institute of Tropical Medicine, Nagasaki University, Japan (Approval No. 10012147).

A 657 bp fragment of env region was amplified by nested PCR. The first reactions were performed in 20 μl volumes containing 1 μl (ca. 50 ng) of the extracted DNA, 200 μM (final conc.) of dNTP mixture, 0.25 μM (final conc.) of the primer sets, 2 μl of 10 × Ex Taq Buffer and 0.5U TaKaRa Ex Taq HS (TAKARA BIO Inc., Shiga, Japan). The external primers were TAATAGCCGCCAGTGGAA AG (nucleotide positions according to the J02029 sequence: 5027–5046) and AGTCCTTGGAGGCTGAACG (6786–6768). The thermal conditions were as follows: 5-min denature at 94°C, 40 cycles of 40 sec at 94°C, 30 sec at 61°C and 40 sec at 72°C, and 10-min final extention at 72°C. The second reactions were performed in 40 μl volumes containing 2 μl of the first PCR product, 200 μM (final conc.) of dNTP mixture, 0.25 μM (final conc.) of the primer sets, 4 μl of 10 × Ex Taq Buffer and 1U TaKaRa Ex Taq HS. The internal primers were CTCCCTTCTAGTCGACGCTCCAGG (5685–5708) and CGTCTGTTCTGGGCAGCATA (6341–6322). The thermal conditions were as follows: 2-min denature at 95°C, 35 cycles of 20 sec at 95°C, 20 sec at 58°C and 30 sec at 72°C, and 2-min final extention at 72°C.

All of the 35 samples from Hokkaido, all of the four from Iwate, 28 of 30 from Kochi, 44 of 50 from Hirado, 21 of 23 from Kumamoto, 46 of 50 from Okinawa and all of the five from Yaeyama were well amplified. RFLP reactions were performed using endonucleases *HpaI* and *HinfI* as designed by Yang et al. [7]. The digested DNA fragments were electrophoresed on 2% agarose gel pre-stained with ethidium bromide and visualized.

RESULTS AND DISCUSSION

All except one of the HTLV-1 isolates from Iwate, Hirado and Kumamoto were determined as the Japanese subgroup, while 20–35% of the isolates from Hokkaido, Kochi, Okinawa and Yaeyama were determined as the transcontinental subgroup (Fig. 1). The electrophoresis profile of two isolates (Hokkaido and Kochi) was consistent with neither the Japanese nor the transcontinental subgroup but similar to the West African/Caribbean subgroup shown by Yang et al. [7]. Thus, these were tentatively treated as "undetermined" in the present paper.

The uneven distribution of the transcontinental and Japanese subgroups in the endemic areas of Japan was clarified in the present study, whereas only the transcontinental subgroup was reported from neighboring areas such as Nogliki of Sakhalin, Kinmen, and Fujian [9–11].

The northern part of mainland Kyushu, represented by Hirado and Kumamoto, seems to be monopolized by the Japanese subgroup. On the other hand, the presence of the transcontinental subgroup ranges from 20 to 35% in the Pacific coast areas of Shikoku (Kochi), the Ryukyu Archipelago (Kakeroma [13] and Okinawa) and Taiwan [7]. An interesting finding in the present study is the presence of the transcontinental subgroup in Kochi, suggesting the endemicity of the transcontinental subgroup along the Kuroshio Current.

A north-flowing ocean current on the west side of the Pacific Ocean, the Kuroshio Current has played the role of an aorta for migration and transportation along the Pacific coast of southwestern Japan since prehistoric times. The endemicity of the transcontinental subgroup along the Kuroshio Current might reflect this human movement. If so, we need to pay more attention to the date and mode of local human movements which may have implications in the epidemiology of HTLV-1 and other infectious agents such as hepatitis B virus [14].

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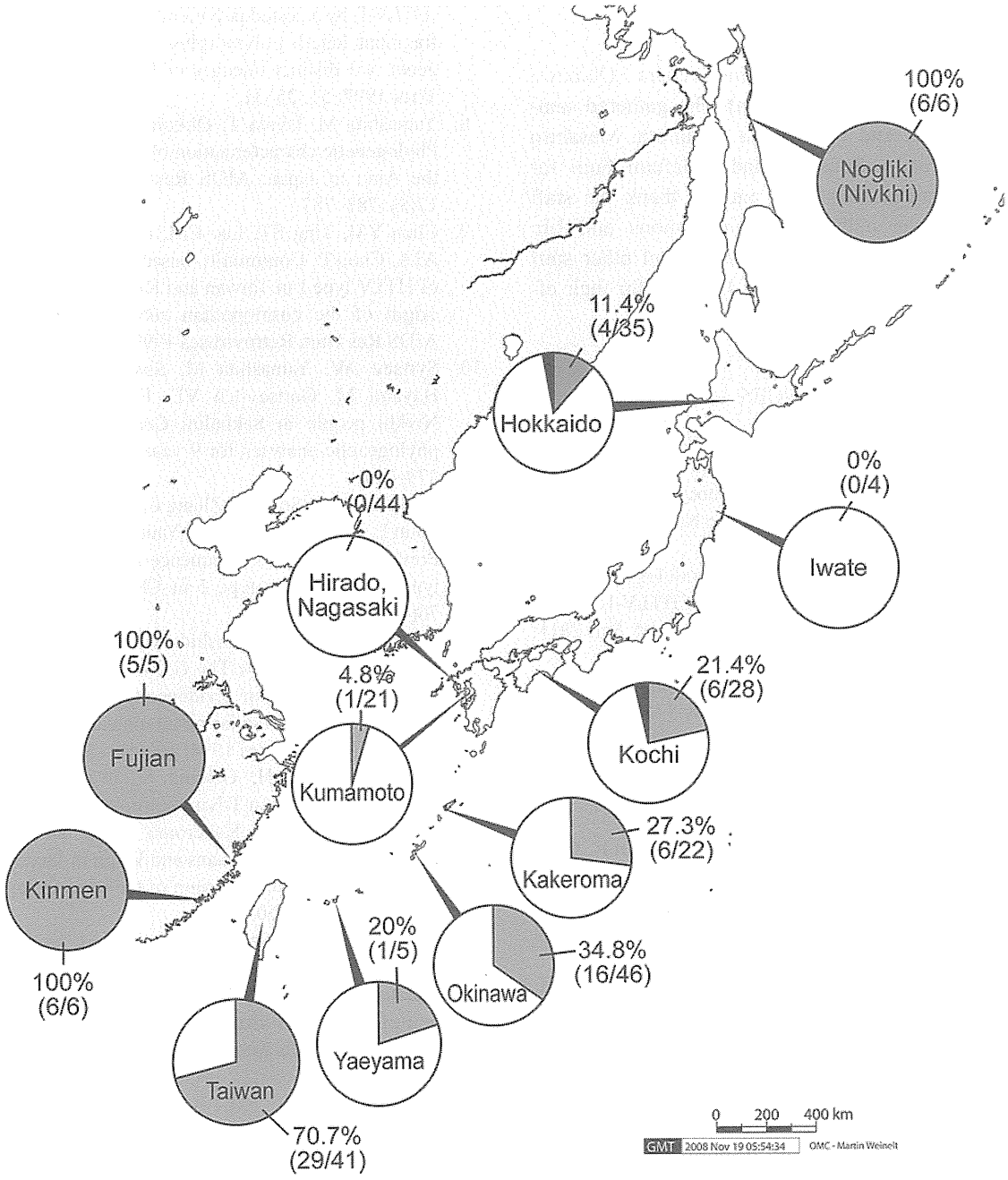

Fig. 1. Ratio of the transcontinental subgroup (grey) to the Japanese subgroup (white) of HTLV-1 cosmopolitan subtype in various localities of East Asia. The data of Nogliki, Kakeroma, Taiwan, Kinmen and Fujian were cited from Syrtsev et al. [10], Eguchi et al. [13], Yang et al. [7], Chen et al. [9] and Wang et al. [11], respectively.

versity. No sponsor, however, participated in the study design, in the collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication.

AUTHOR DISCLOSURE STATEMENT

Drs. Otani, Yamamoto and Eguchi have full access to all the data in the study and hold final responsibility for the decision to submit for publication. All authors declare that they have no conflict of interest.

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I 造血器腫瘍の疫学

成人T細胞白血病・リンパ腫/HTLV-1感染の疫学
—HTLV-1感染症の根絶に向けて—

Epidemiology of adult T-cell leukemia and HTLV-1 infection
—Toward the eradication of HTLV-1 infectious diseases—

山口一成

Key words：HTLV-1，成人T細胞白血病・リンパ腫(ATL)，疫学，HTLV-1キャリア

1 ATL/HTLV-1の現状

我が国には人口の1％にヒトT細胞白血病ウイルスI型(HTLV-1)感染者(キャリア)が存在しており，年間1,000人以上が死亡する成人T細胞白血病・リンパ腫(ATL)をはじめとするHTLV-1関連疾患の発症予防や治療法の開発は急務である[1,2]．

2010-11年にかけて，HTLV-1に関連して大きな動きがあった．厚生労働省の研究班が20年ぶりにHTLV-1の感染者，およびATL，HTLV-1関連脊髄症(HAM)の全国調査を行い，現時点での全国の推定キャリア数は108万人と，1980年代の120万人から減少はみられるものの引き続き多くの感染者が存在していたことを明らかにした(図1)．また地域別割合では，九州・沖縄のキャリア数の割合は減少しているものの，関東は増加しており，感染が大都市圏に拡散していることが示唆された[3,4]．

ATLの年間発症数は20年前の700人/年から，現在は1,146人と推測された．ATL患者の男女比は1.16と男性にやや多く，患者発症年齢の中央値は67歳であり，第9次ATL全国実態調査の結果(平均61歳)と比較してこの20年で約10歳近く高齢化が進んでいた．我が国における高齢者を中心とした巨大なキャリアのプールから，今後も持続的にATLは発症し，患者はますます高齢化すると推測された．

a. HTLV-1関連疾患患者数

ATL，HAMの患者数の全国調査を行うため，各地域ごとに患者数が多いと考えられる代表的な病院へ協力依頼を行った．調査する病院では，HTLV-1感染とは無関係でかつ国内における地域別の有病率が既に知られている血液悪性腫瘍，神経疾患についてもその患者数を同時に調査し(内部コントロール)，それぞれに対する割合を表すことで調査の精度を高めた．この結果にキャリアの地域分布を重ね合わせ，キャリアからのHTLV-1関連疾患の発症率を推測した．

1) ATLの全国実態調査

ATLの全国調査(2006-07年までの2年間に発症)では，報告された154施設の患者数は，ATL 902例(11.4％)，B細胞性非ホジキンリンパ腫(B-NHL：内部コントロール)7,008例(88.5％)．九州・沖縄では全国のATLの60％，B-NHLは20％で，ATL発症の大きな地域差が再確認された．ATL患者数は20年前に比べて確実に増加していた．

今回の調査は2年間の調査であるため，ATLが455例に対しB-NHLは3,582例/年間の比率

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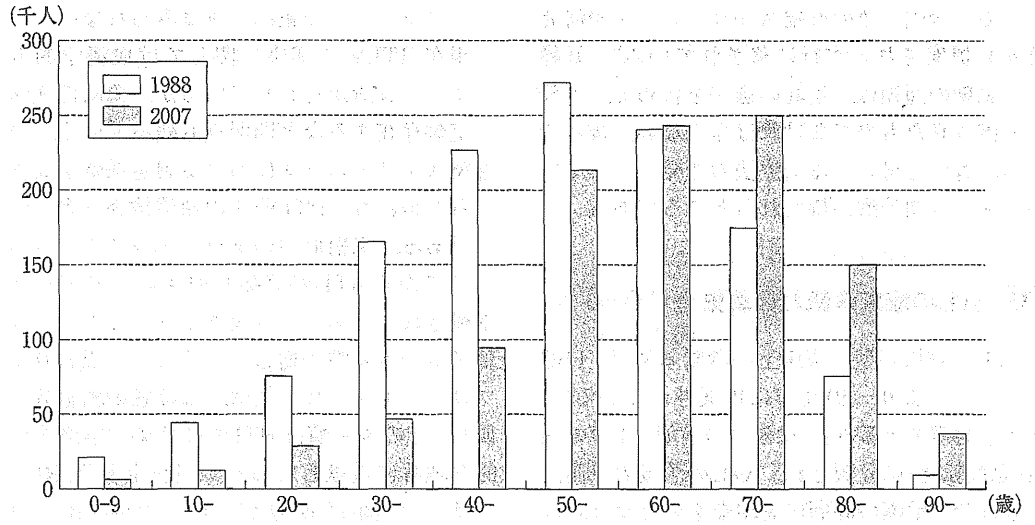

図1　推定されるキャリアの年齢別分布の推移(文献[3]より引用)
1988年：120万人，2007年：107万9千人.

で発症したことになる．これらの数値をもとにATLの年間発症数を推測してみた．厚生労働省の人口動態調査(死因分類)によれば，2006-08年のATLによる死亡数は平均1,056例/年であるのに対し，B-NHLによる死亡数は平均9,020例/年である．発症と死亡の比率がATLとB-NHLで同程度と仮定し，B-NHLをコントロールとして計算すると，ATLの年間発症数は1,146例となる．

ATL患者の高齢化が顕著に進んでおり，ATLに対する根治的治療法として同種造血幹細胞移植療法が行われているが，ミニ移植を除くと対象年齢の上限はおおよそ55歳である．今回の患者年齢の分布をみると，55歳未満は全体の19.2％にすぎない．第9次ATL全国実態調査では55歳未満が32.5％であり，10年間で55歳未満の症例が13％も減少したことになる．今後も移植が行える症例は持続的に減少していくと推測され，治療戦略の変更を余儀なくされると思われる．

この人口動態調査でATL発症数の年次推移を過去10年間遡ってみると，ATLによる死亡数は過去10年間全く減少していない．

2) HAMの全国実態調査

HAMについても新規に発症し診断される患者は増加傾向にあり，患者は九州以外の大都市でも多くみられた．

HAMの全国調査は，1987年に初めて行われ，710例が報告され，1994年は確実例1,103例，1998年は擬診例を含めて1,422例と報告された．平均年齢は63.0歳，男女比は1：2.5で女性が多く，発症時年齢は49.4歳である．HAMの推定有病率は人口10万人あたり3人であった．

1994年前後の診断のHAM患者を比較すると，65歳以上の高齢発症者は1994年以前が2.5％(4/161例)に対し，1995年以降で22.5％(129/574例)に増えており，高齢発症者の増加がうかがえる．また，患者の分布では大都市部では，1994年以前で関東28例(17.4％)，近畿13例(8.6％)であったのが，1995年以降にはそれぞれ関東194例(30.3％)，近畿67例(11.6％)と増えていた．2004年以降は毎年50人以上が新規に診断されている．

これらの結果はHTLV-1が決して'消えゆくウイルス'ではなく，むしろ全国的にはキャリア，患者の分布が地方から都会へと拡散していることが示された．そして2つの問題を提起している．1つは全国での感染防止対策，もう1つは高齢者のATL/HAM対策である．前者ではHTLV-1の主な感染ルートである母子感染対策

として，全国一律の妊婦スクリーニングが研究班から提案され，実行に移されている[5]．妊婦への感染の通知は，授乳の選択を含めて，十分に配慮されたものでなければならない．後者では高齢者にも耐えうる治療法の開発，そして早期診断・早期治療が待たれるところである．

2 ATLの臨床病態と自然史

ATLの臨床病型，臨床上の診断基準は，LSGグループにより，1991年に提案されている[6]．様々な病態をとるATLは4つの病型，1つの病態(急性転化)に分類され，ATLの予後の予測，治療方針，治療の評価に有用なものとなり，国際的にも汎用されている．

HTLV-1は感染後，ウイルスRNAから逆転写酵素の働きでDNAを合成し細胞の染色体DNAに組み込ませる(プロウイルス)が，その組み込み部位はランダムである．キャリアの感染細胞はポリクローン性増殖から，モノクローン性増殖へと移行し，ATLを発症する．その中間状態の患者からATLの発症がみられており，ウイルス感染細胞の増加した状態はATLの発症とも密接に関連している．HTLV-1感染細胞を増やす機序とそれを排除しようとする免疫学的機序のせめぎ合いの中で，感染細胞はポリクローナルに増殖し，更にその一部がモノクローナルになり，更に遺伝子異常が蓄積して，悪性転換し，ATLが発症する．

3 HTLV-1抗体検査とHTLV-1プロウイルスの遺伝子診断 —ウイルス量測定の標準化へ

HTLV-1抗体はATL，HAM，HTLV-1ぶどう膜炎などのHTLV-1関連疾患の診断，母子感染や輸血による感染防止のためのキャリアを同定するのに不可欠である．

HTLV-1抗体陽性で臨床的にATLと診断された症例は，その腫瘍細胞に全例HTLV-1プロウイルスのモノクローナルな組み込みがみられる．しかし健康人キャリアではプロウイルスのモノクローナルな組み込みはみられない[7]．

現在HTLV-1感染に関しては血清学的方法によって診断が行われているが，偽陽性検体が一定数存在するなど問題点も残っている．母子感染スクリーニングにおける判定保留を改善させるためにも，HTLV-1の核酸検査は課題の一つである．末梢血中のプロウイルス量の多いキャリアからATLが発症しやすいとのデータも蓄積されつつあり，今後キャリアでのウイルス量測定の重要性が増してくるものと思われる．プロウイルスコピー数は，ほぼ感染細胞数に近似し，その測定値はHTLV-1感染の病態生理学的な理解に貢献しており，現在定量PCR(ポリメラーゼ連鎖反応)法についての再評価，測定系の標準化が進められており，近い将来には良質で安定した標準品を供給することが可能であろう[8]．

4 HTLV-1の感染ルートについての話題

(1) ATL発症に直接結びつくと考えられている母乳を介した母子感染：母親がキャリアの場合，その子どもは15-30％が感染する．これを遮断する試みとして，キャリアの母乳を中止することが行われている．しかし断乳した場合でも2-3％の児に感染が成立しており，また短期間の母乳投与は人工乳投与と差がないという結果もあるため，研究班が発足している．

(2) 精液中のリンパ球を介する夫婦間感染(主に夫から妻へ)：成人後の感染によりATLが発症することは極めてまれであり，現実に感染を予防することが困難であることから放置されているが，従来考えられていたよりも男女間の感染は高い頻度で起こっている可能性が示唆されている．

(3) 輸血：献血者に対するスクリーニングは1986年から開始されている．スクリーニング以前には多くの患者が輸血によりHTLV-1に感染したと推定されるが，現在は完全に防げている．スクリーニング以前には輸血によるHAMの発症の報告が多数あったが，ATLにつ

いての報告は極めてまれである.

現在，献血者でのHTLV-1抗体陽性率は年々減少しており，特に若い世代ほど抗体陽性率は年々低くなっている．この自然減少の理由として，戦後人工栄養が増え母乳が急激に減ったこと，しかも母乳栄養の期間が著しく短くなっていること，子どもの数の減少，性行動の変化などの複合的なことが考えられている.

5 HTLV-1の分布と起源

世界でもHTLV-1は偏在している．カリブ海沿岸諸国，南米，イラン，イスラエル，アラスカ出身のアリュート人，イタリア，アフリカ中央部，西ヨーロッパ(英国，フランス，オランダなどのカリブ海諸国からの移民)などにキャリア，患者が分布している.

HTLV-1はgenotypeとして，コスモポリタン型，中央アフリカ型，メラネシア型などに分類される．HTLV/STLVの遺伝子亜型分類から民族の世界的移動を推定する研究もある.

6 HTLV-1関連疾患(HTLV-1関連脊髄症(HAM/TSP)，HU/HAU，その他)

鹿児島地方に以前から多かった痙性脊髄麻痺を主徴とする神経疾患をHTLV-1関連脊髄症として，そして第3のHTLV-1関連疾患としてHU/HAUが疾患として確立している．HU/HAUは，特に成人若年者に多く，ベーチェット病，トキソプラズマ症など既知のぶどう膜炎とは異なる眼科的臨床像である．突発性に生じる飛蚊症，霧視，軽度の視力低下などの症状を呈し女性に多い．一般に予後は良好であるが，約60％の症例で再発がある．HU/HAUの有病率はキャリア10万人あたり90-110人，女性は男性の2.6倍多い．HTLV-1ぶどう膜炎患者の既往歴として，バセドウ病の頻度が有意に高く，その発症要因に自己免疫機序が関与していると考えられる.

そのほか，関節病変，間質性肺炎やTリンパ球性肺胞炎などの肺病変，シェーグレン症候群，多発性筋炎，慢性腎不全，非特異的リンパ節炎，感染性皮膚炎(小児)などにHTLV-1の関与が考えられているが，疫学的な裏づけは確立していない.

7 ハイリスクキャリアの早期同定を目指したHTLV-1キャリアの前向き研究

キャリアにおけるATL発症要因についてはこれまで明確に同定されていなかったが，多施設共同研究JSPFADプロジェクト[9]により明らかになった．全国の拠点病院で2002-08年の間に1,218例のキャリアをフォロー中に，14例がATLを発症し，この14人の発症前の末梢血中のウイルス量はいずれも高かった．多変量解析により，①ウイルス量，②ATLの家族歴，③年齢，④他の疾患で治療中，などが発症の危険因子として確認された[10].

8 '慢性HTLV-1感染症'の概念

ATLを含むHTLV-1関連疾患の理解に向けて，血液学，ウイルス学，分子生物学，腫瘍学，神経学などで多くの成果を上げてきた．しかしこれまでの研究を振り返ってみると，ATL，HTLV-1関連疾患をHTLV-1感染症としてとらえる視点，その対策が不十分だったのではないかと考える．再度ATLを含めたHTLV-1関連疾患の原点は'感染症'であることを認識し，感染を防止すること，すなわち母子感染の遮断，感染防止ワクチンの開発，HTLV-1キャリア期，'慢性HTLV-1感染症'の時期における積極的な薬物療法の開発などが望まれる.

'慢性HTLV-1感染症'はキャリアの末梢血リンパ球にウイルス感染細胞数が4％以上に増加した状態で，更に何らかの基礎疾患，例えば慢性気管支炎，非特異的リンパ節炎，糞線虫症[11]，関節炎などに罹患している状態を示す新たな疾患概念と定義し，この時期にHTLV-1関連疾患発症を防止する戦略を全力で構築すべき

であろう.

9 今後の展望

ATL/HTLV-1研究は30年が経過した. 今後のHTLV-1感染・関連疾患発症の予防や治療に関する総合的な対策を策定し, それを実践する目的で, ATLおよびHAMの現時点の全国的な実態が明らかにされ, ATL/HTLV-1対策に大きな動きが出てきた. そして新しい研究の芽生えもみえている. 感染予防のための教育・啓蒙や予防法, 治療法の周知・徹底といった医療および行政面からの総合的な取り組みも今後速やかに実践していく必要がある. 今後のHTLV-1感染症への対策として, 国を挙げてのHTLV-1感染症の実態の把握, 病態および発症機構の解明, HTLV-1関連疾患の発症予防, 治療法の開発, 研究基盤の整備などが挙げられる.

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

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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

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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′-GGCTCT AAGCCCCCGGGGGATATTTGGGGCTCATGG-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′-GGCGACTGGTGCCCCATCTCTGGGGGACTATGTTCG-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′-GTGATCCATGGTGTTTGTCCACCTGAAAGC-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.

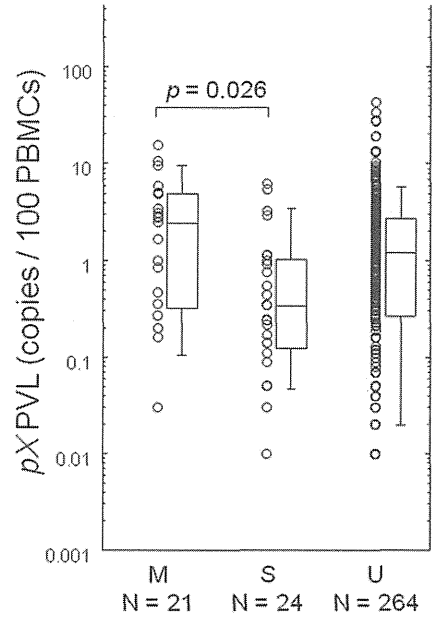

Figure 1. *PX* PVLs in HTLV-1 carriers with different infection routes M: Carriers with infection from mothers; S: Carriers with infection from spouses; U: Carriers with undetermined infection routes.

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.

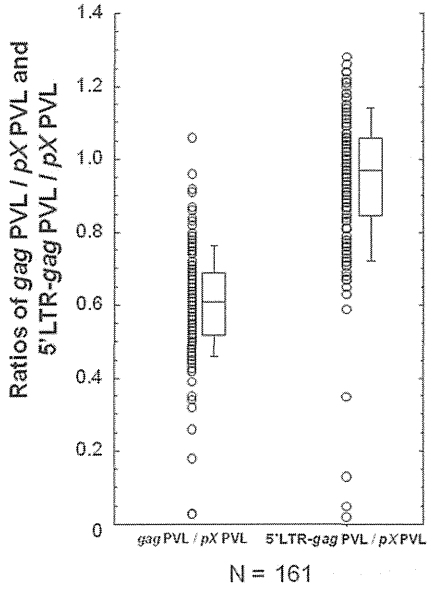

Figure 2. The ratios of PVLs for the 5′LTR-*gag* or *gag* regions to PVL for the *pX* region in 161 asymptomatic HTLV-1 carriers, whose *pX* PVLs were equal to or greater than 1 copy/100 PBMCs.

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

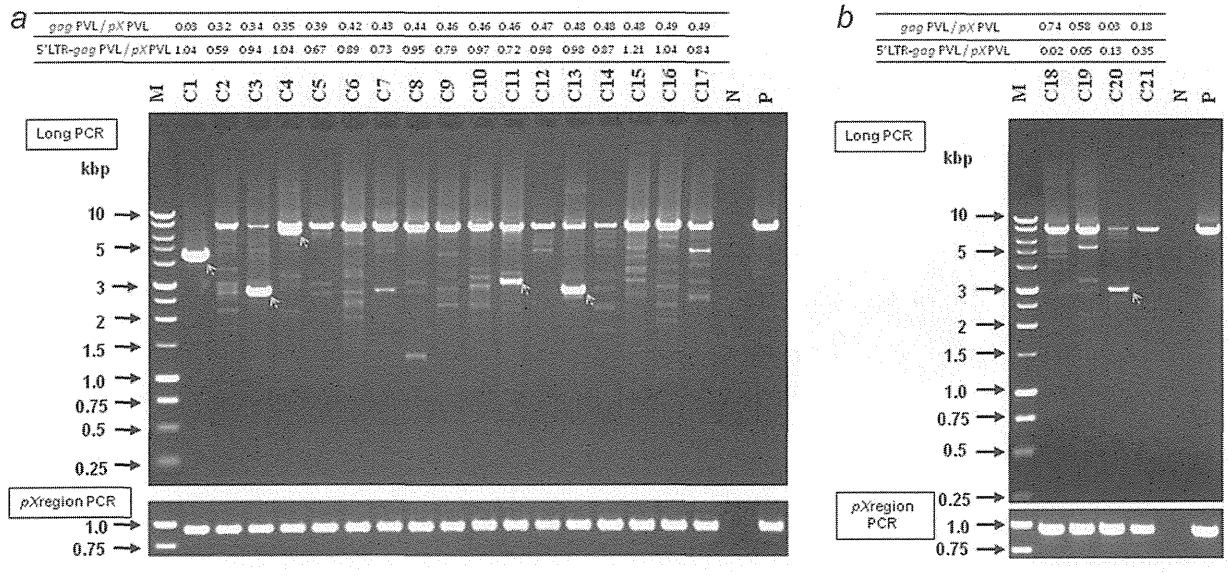

Figure 3. Detection of defective provirus by long PCR. (*a*) Asymptomatic HTLV-1 carriers with low *gag* PVL/*pX* PVL ratios less than 0.5. (*b*) Asymptomatic HTLV-1 carriers with low 5′LTR-*gag* PVL/*pX* PVL ratios less than 0.5. Arrows indicate PCR products for HTLV-1 provirus lacking large internal 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*).

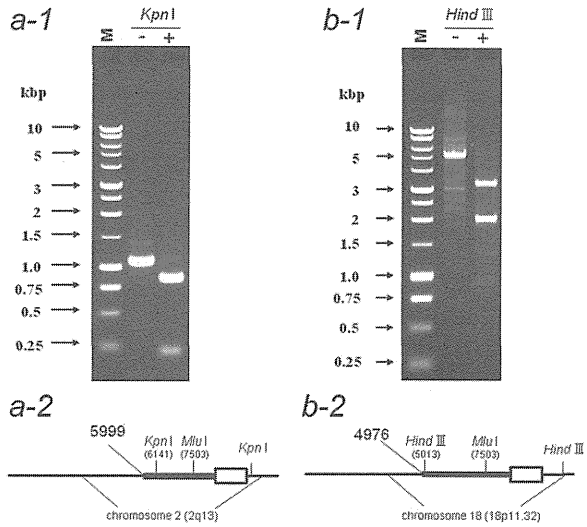

Figure 4. Detection of provirus with deletion of 5′LTR and its internal flanking sequence by IL-PCR. (*a-1*) Long PCR products from an asymptomatic HTLV-1 carrier, C20, with or without *Kpn* I digestion. (*a-2*) Scheme of the structure of defective provirus in C20. (*b-1*) Long PCR products from an asymptomatic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

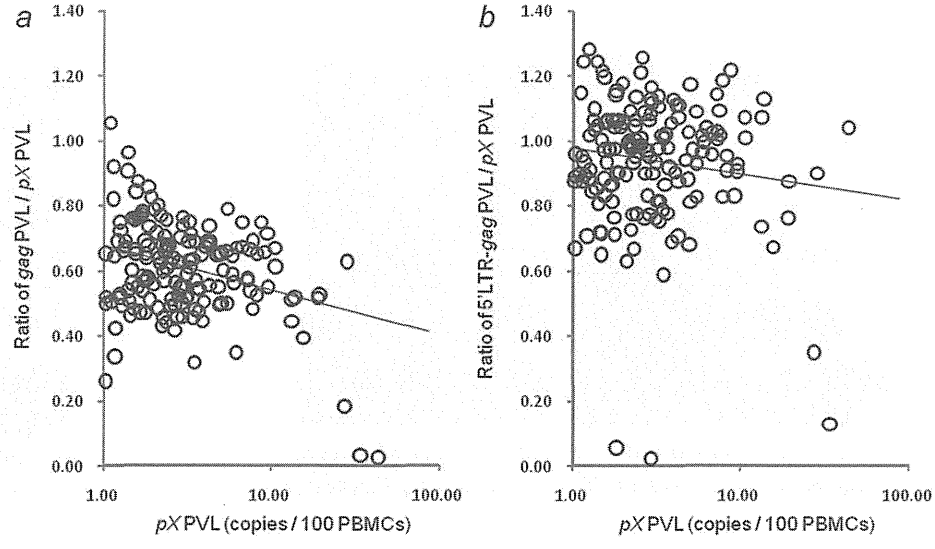

Figure 5. Relations of *pX* PVL and *gag* PVL/*pX* PVL or 5′LTR-*gag* PVL/*pX* PVL ratios in 161 asymptomatic carriers. (*a*) Relation of *pX* PVL and *gag* PVL/*pX* PVL. (*b*) Relation of *pX* PVL and 5′LTR-*gag* PVL/*pX* PVL.

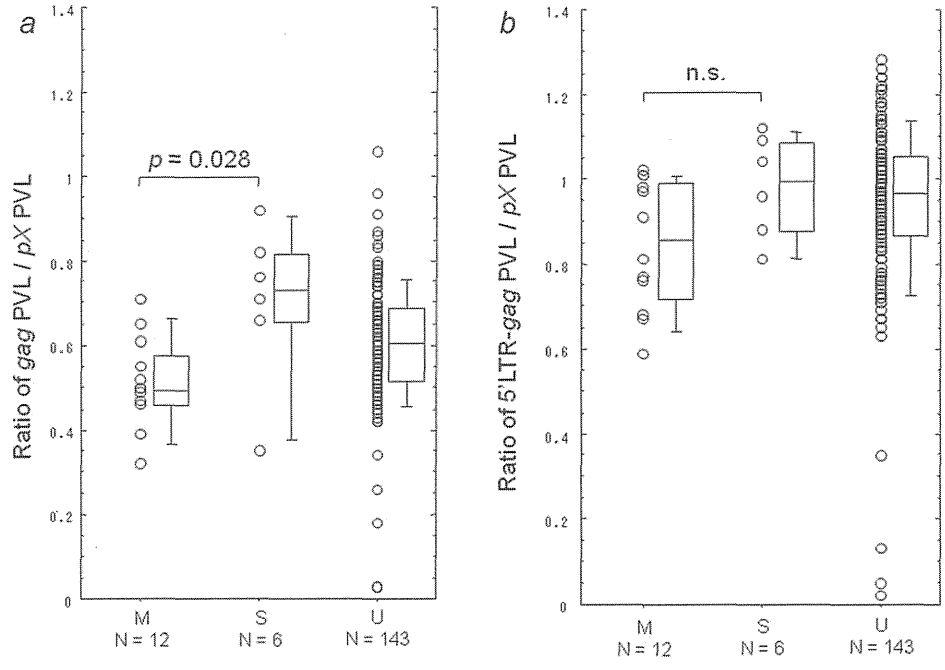

Figure 6. The ratios of *gag* PVL/*pX* PVL or 5′LTR-*gag* PVL/*pX* PVL in HTLV-1 carriers with different infection routes in 161 asymptomatic carriers. (*a*) The ratio of *gag* PVL/*pX* PVL. (*b*) The ratio of 5′LTR-*gag* PVL/*pX* PVL. M: Carriers with infection from mothers; S: Carriers with infection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

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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