Table 2 | Risk factors for the development of ATL with regard to the HTLV-1 carrier status.

	Reference
Host susceptibility	
Vertical infection with HTLV-1 as infant	Murphy et al. (1989)
Attained at an age of >50 years	Many references
Male sex	Many references
HLA-A*26, HLA-B*4002, HLA-B*4006, and HLA-B*4801 (Japanese ATL)	Yashiki et al. (2001)
Co-infected with *Strongyloides stercoralis*	
Laboratory markers	
A high level of sil-2R, more than 500 U/ml	Arisaw et al. (2002)
A high level of anti-HTLV-1, titer more than × 1,024	Arisaw et al. (2002)
A high level of circulating abnormal lymphocytes, more than 0.6%	Hisada et al. (1998a)
A low level of of anti-Tax reactivity	Hisada et al. (1998b)
A high level of white blood cell count, more than 9,000/μL	Imaizumi et al. (2005)
Viral markers	
A higher HTLV-1 proviral load level, more than 4 copies per 100 PBMCs	Iwanaga et al. (2010)

ATL, adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; HLA, human leukocyte antigen; PBMC, peripheral blood mononuclear cell; sIL-2R, soluble interleukin-2 receptor.

AFRICA AND EUROPE

In Africa, a high HTLV-I seroprevalence rate (>2% in the adult population) has been reported in sub-Saharan African countries, especially in Gabon (Hunsmann et al., 1984; Delaporte et al., 1988; Gessain, 1996; Etenna et al., 2008; Gonçalves et al., 2010). Although there are many reports regarding the HTLV-I seroprevalence rates in African countries, only a few epidemiological studies of ATL were available. In a case-control study including NHL and control that performed in Gabon, only four cases of the 26 patients with NHL fitted the criteria of ATL (Delaporte et al., 1993), but further information on epidemiological feature of ATL was not available.

In Europe, HTLV-1 is endemic in Southern Italy (Manzari et al., 1985). Several case series of ATL were reported from Europe (Manzari et al., 1985; Gessain et al., 1990). Most of ATL patients were African origin from high-HTLV-1-endemic areas (West Indies, Nigeria, and other African areas); however, some patients had no background regarding endemic areas (Manzari et al., 1985).

RISK FACTORS FOR ATL IN HTLV-1 CARRIERS

Although a variety of genetic abnormalities due to HTLV-1 infection have been reported to explain the characteristics of ATL oncogenesis, HTLV-1 infection alone is not sufficient to develop ATL from HTLV-1 carrier status. Risk factors for developing ATL in HTLV-1 carriers have been investigated in many epidemiological and clinical studies (**Table 2**).

HOST SUSCEPTIBILITY

Age is a well-known risk factor for the development of ATL. ATL occurs mostly in adults, at least 20–30 years after HTLV-1 infection. However, the age at onset differs across geographic areas, which may be affected by racial or environmental characteristics. In Japan in the early 1980s, an average age at diagnosis of ATL was reported to be individuals in their early 1950s (The T- and B-Cell Malignancy Study Group, 1981, 1985), but the age at diagnosis increased yearly, reaching 65 years in the latest nationwide survey for ATL (Yamada et al., 2011). However, the average age at diagnosis of ATL in Jamaican and Brazilian series was reported to be individuals in the 1940s (43 years in Jamaica and 44 years in Brazil; Hanchard, 1996; Pombo de Oliveira et al., 1999), which is younger than that in Japan (Yamaguchi et al., 1987).

The age at the time of HTLV-1 infection is also a very important risk factor for the development of ATL. Individuals infected in childhood (vertical transmission) may be at higher risk for developing ATL (Murphy et al., 1989). ATL seldom develops in individuals infected in adulthood, although no epidemiological study has proven this fact. There was one case report describing that a female HTLV-1 carrier known as conclusively transmitted horizontally by her partner developed ATL (Sakuma et al., 1988). To clarify whether or not ATL develops among individuals infected in adulthood, a large prospective follow-up study is required.

Male sex is considered a risk factor for ATL. In most studies from Japan, the incidence of ATL is two- and threefold higher in male carriers than in female carriers, which is contrary to the higher rate of HTLV-1 positivity in women than in men. However, a population-based survey in central Brooklyn reported that the annual incidence of ATL was higher in women than in men (male-to-female ratio of 1:3; Levine et al., 1999). Modeling data from Jamaican series also showed a higher cumulative lifetime risk of ATL in women than in men (4.0% for men and 4.2% for women; Murphy et al., 1989). The reason for the sex-related differences in the incidence rate of ATL between Japan and other regions is unknown.

It seems unlikely that there are apparent ethnical differences in susceptibility to infection by HTLV-1 and developing ATL. A higher incidence of ATL was found individual of African origin than in others (Manzari et al., 1985; Gessain et al., 1990; Yamamoto and Goodman, 2008), however, most of patients of African origin came from HTLV-1 endemic areas.

Earlier epidemiologic studies have found that ATL patients are more likely to have a family history of lymphoid malignancy (Ichimaru et al., 1979; The T- and B-Cell Malignancy Study Group, 1981). Since then, several host genetic background factors influencing the onset of ATL have been investigated. Human leukocyte antigen (HLA) is a candidate for the genetic factors controlling the immune response against the viral antigen. Specific HLA antigen alleles have been reported to be associated with an increased risk of developing ATL (Uno et al., 1988). The allele frequencies of HLA-A*26, HLA-B*4002, HLA-B*4006, and HLA-B*4801 were significantly higher in ATL patients than in asymptomatic HTLV-1 carriers in southern Japan, and ATL patients possessing these alleles developed ATL 12.6 years earlier than patients with other alleles (Yashiki et al., 2001). Ethnic differences in HLA alleles related to ATL were also investigated in another study (Sonoda et al., 2011).

HTLV-1 carriers with abnormal immune system may be at high-risk of developing ATL. Several studies reported that HTLV-1 carriers co-infected with *Strongyloides stercoralis* are considered

a high-risk group for developing ATL because of the clonal proliferation of HTLV-1-infected lymphocytes and high proviral load (Nakada et al., 1987; Yamaguchi et al., 1988; Plumelle et al., 1997; Gabet et al., 2000). Satoh et al. (2002) suggested that *S. stercoralis* infection induces polyclonal expansion of HTLV-1-infected cells by activating the interleukin 2/interleukin 2 receptor (IL-2/IL-2R) system in dually infected carriers, which may be a precipitating factor for ATL. The immunosuppressive state has been reported to potentially contribute to ATL development in HTLV-1 carriers. There were several case reports of ATL developed in HTLV-1 carriers undergoing immunosuppressive treatment after living-donor liver transplantation (Kawano et al., 2006; Yoshizumi et al., 2012) and kidney transplantation (Hoshida et al., 2001).

LABORATORY MARKERS

Several laboratory abnormalities were found to be markers for the development of ATL. Kamihira et al. (1994) measured prospectively soluble IL-2R (sIL-2R) levels and lactate dehydrogenase (LDH) levels in HTLV-1 carriers, reporting that the increasing level of sIL-2R may be a more sensitive indicator of ATL than LDH. A nested case-control study also showed that high levels of sIL-2R (more than 500 U/mL) and HTLV-1 antibody titers (more than 1,024) were independently associated with an increased risk of developing ATL (Arisaw et al., 2002). Imaizumi et al. (2005) analyzed the outcomes of 50 HTLV-1 carriers with monoclonal proliferation of HTLV-1-infected T cells in a 20-year follow-up study, reporting that a high white blood cell count more than 9,000/μL was a potential prognostic factor for developing ATL, even after adjustment for age, sex, and relative lymphocyte counts.

A series of the Miyazaki Cohort Study (population size; 1,960 people, of whom 27% were HTLV-1 antibody-positive) reported that an HTLV-1 carrier with a high anti-HTLV-1 titer (odds ratio; 1.6), a high number of circulating abnormal lymphocytes, and a low anti-Tax reactivity were associated with a greater risk of developing ATL (Mueller et al., 1996; Hisada et al., 1998a,b). Recently, an international ATL Cohort Consortium study by merging eight cohorts from Japan, Jamaica, the United States, and Brazil examined serologic markers of HTLV-I pathogenesis and host immunity in 53 ATL cases and 150 matched asymptomatic HTLV-I carriers (Birmann et al., 2011). The study confirmed that above-median sIL-2R and anti-Tax seropositivity were independently associated with an increased ATL risk, and found that above-median total immunoglobulin E levels predicted a lower ATL risk.

Aberrant expression of cell-surface antigens is usually used for clinical routine diagnosis on ATL. ATL cells phenotypically express CD4, CCR4, and CD25. However, data of cell-surface antigens rarely used for a prognostic marker of ATL from HTLV-I carriers. Two studies reported that expression of CD3, CD7, and CD26 on HTLV-1-infected cells were diminished in acute and chronic ATL and those were slightly down-regulated in smoldering ATL (Tsuji et al., 2004; Tian et al., 2011). These results suggest that the down-regulation of those cell-surface antigens could be possible predict markers for the early phase leukemogenesis of ATL from HTLV-1 carriers. A resent study serially evaluated cell-surface antigens on HTLV-1-infected cells in HTLV-1 carriers, smoldering ATL, and chronic ATL, by taking into consideration the pattern of Southern blot hybridization and proviral load (Kamihira et al., 2012). The report suggests that the decreasing expression of CD26 and the decreasing ratio of CD26/CD25 are novel biomarkers for prediction of clonal bands and discrimination of carriers and smoldering ATL.

PROVIRUS-INTEGRATION STATUS

Among HTLV-1 carriers, there exist a group of cases having the monoclonal integration of HTLV-1 proviral DNA in mononuclear cells without signs of malignant proliferation or clinical signs and symptoms related to leukemia (Ikeda et al., 1993). Such carriers have been suggested to be a high-risk group of developing ATL, but their prognosis varied from being stable carriers for long to developing ATL (Ikeda et al., 1993; Imaizumi et al., 2005). There are only a few epidemiological studies to investigate the significance of the provirus-integration status on non-malignant infected cells from asymptomatic HTLV-1 carriers.

Nakada et al. (1987) reported that patients with *S. stercoralis* infection and co-infected with HTLV-1 had a high frequency (35%) of patients presenting a monoclonal integration of HTLV-1 proviral DNA in their blood lymphocytes. Carvalho and Da Fonseca Porto (2004) also The author also found a correlation between monoclonal integration of proviral DNA and abnormal lymphocytes in peripheral blood, with a trend for greater severity of the parasitic infection. Although several studies reported that HTLV-1 carriers co-infected with *S. stercoralis* are considered a high-risk group for developing ATL (Nakada et al., 1987; Yamaguchi et al., 1988; Plumelle et al., 1997; Gabet et al., 2000), no study investigated the clinical significance of the monoclonal integration of HTLV-1 proviral DNA in their blood lymphocytes in HTLV-1 carriers with *S. stercoralis*.

PROVIRAL LOAD

In the area of viral oncogenesis, there are accumulated data indicating a relationship between an increased viral load and viral-associated malignancies. HTLV-1 proviral DNA load in the peripheral blood mononuclear cells (PBMCs) are also evaluated in some epidemiological and clinical studies to support the hypothesis that increased HTLV-1 proviral load level is an important predictor of developing ATL.

A cross-sectional study (Manns et al., 1999) and a series of the Miyazaki cohort study (Tachibana et al., 1992; Hisada et al., 1998a,b; Okayama et al., 2004) reported that HTLV-1 proviral load level was higher in HTLV-1 carriers who developed ATL than in asymptomatic HTLV-1 carriers. However, the proviral load was measured only in a small number of subjects in the above literature.

Several large-scale prospective studies support results from the previous small studies that an increased HTLV-1 proviral load is an important predictor of developing ATL. In Japan in 2002, a nationwide prospective cohort study for asymptomatic HTLV-1 carriers, the Joint Study on Predisposing Factors of ATL Development (JSPFAD), was initiated (Yamaguchi et al., 2007) to investigate viral- and host-specific determinants of the development of ATL in more detail. In the cohort of 1,218 asymptomatic HTLV-1 carriers (426 men and 792 women), 14 subjects progressed to overt ATL during a follow-up of 1981.2 person-years (Iwanaga et al., 2010). All of the 14 subjects were among those with the highest group of baseline proviral load (range, 4.17–28.58 copies/100

PBMCs). Multivariate Cox analyses indicated that a higher proviral load (more than 4 copies/100 PBMCs) is an independent risk factor for progression of ATL, even after adjusting for sex, age, family history of ATL, and other possible risk factors. The result indicated that HTLV-1 carriers with higher HTLV-1 proviral load levels belong to the high-risk group of carriers who develop ATL and in whom any measures to prevent the development of ATL should be instituted.

Nevertheless, the association between HTLV-1 proviral load and disease development remains unclear because a higher proviral load is also an important predictor in patients with HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Further viral markers are needed to determine the function of a higher HTLV-1 proviral load to direct the way to developing ATL or developing HAM/TSP from HTLV-1 carriers.

CONCLUDING REMARKS

Although many prior studies found important epidemiological evidence on ATL and risk factors for the development of ATL in HTLV-1 carriers, limited data are available on the valid annual incidence of ATL from longitudinal prospective studies. Existing predisposing factors are still insufficient to explain the characteristics of ATL oncogenesis. Unknown risk factors may be involved in the acquisition of malignant characteristics of HTLV-1 infected cells. Further well-designed epidemiological studies are needed to fully understand the oncogenesis of ATL.

Even though the incidence of ATL is relatively low among HTLV-1 carriers and a novel promising agent, mogamulizumab (humanized anti-CCR4 monoclonal antibody), is released (Ishida et al., 2003, 2012), preventing new HTLV-1 infections and the development of ATL are major public health concerns in HTLV-1 endemic countries in the world. In Japan, there are approximately one million of HTLV-1 carriers, 1,000 new ATL cases, and 1,000 new deaths from ATL every year. However, only recently has the Japanese government for the first time begun to implement a nationwide comprehensive package of measures covering the prevention of mother-to-child HTLV-1 transmission and the development of medical researches on HTLV-1 and ATL (http://www.kantei.go.jp/foreign/kan/actions/201009/13htlv_e.html). The challenge in the next few years will be to reduce the number of HTLV-1 carriers, to develop an easy method that allows identification of high-risk carriers, and to implement earlier therapeutic interventions for carriers with high-risk markers.

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【ATLの疫学 現状と課題】

Epidemiology of ATL : Current knowledge and future challenges

岩永 正子

Masako Iwanaga

Key words:
HTLV-1キャリア，ATL，疫学，
リスクファクター，プロウイルス量

要 約

HTLV-1感染者が多い地域は偏在しており，世界的には日本，カリブ海沿岸諸国，南米，など，日本国内では九州，沖縄などである。日本には約108万人のHTLV-1キャリアが存在し，毎年約1100人以上のATLが発症し，毎年約1000人がATLにより死亡している。HTLV-1キャリアからの年間ATL発症率は，1,000人あたり男1～1.5，女0.5～0.7，30歳以上のHTLV-1キャリアにおける生涯ATL発症率は，男4～7%，女2～5%と推定されている。ATL発症のリスクファクターとして，母子間感染，男性，加齢，特定のHLA保持者，免疫低下などの宿主側要因や，感染細胞数の多さを反映する白血球増加，異型リンパ球増加，ウイルス抗体価上昇，可溶性IL-2R上昇などの臨床検査値異常や，HTLV-1プロウイルス量の上昇などが報告されている。しかし，ATL発症のリスクファクターの全容解明にはいたっておらず，遺伝的背景，免疫学的背景，分子生物学的特徴などを含むさらなるエビデンスの集積が必要である。

はじめに

白血病やリンパ腫の原因は大半が不明であるが，成人T細胞性白血病・リンパ腫（Adult T-cell Leukemia-lymphoma, ATL）は，ヒトT細胞性白血病ウイルス（Human T-cell Leukemia Virus type I, HTLV-1）の持続感染者（HTLV-1キャリア）からのみ発症するウイルス起因性の血液悪性腫瘍である。本稿では疫学的観点から，HTLV-1キャリア数，ATLの発症数や死亡数，HTLV-1キャリアからのATL発症率，発症にかかわるリスクファクターについてこれまでの知見を紹介し，さらに何がまだ未解決の課題として残されているのかを解説する。

1．HTLV-1キャリアの分布と推計数

世界的にみても日本国内でも，HTLV-1キャリアの分布は極めて偏在している。日本，カリブ海沿岸諸国，南米，アフリカ中央部，パプアニューギニア，オーストラリアのアボリジニやアフリカのピグミー族，北米および南米の先住民族，イランの一部の地域などが，HTLV-1感染者の割合が高い地域として知られている（図1）[1]。日本国内でHTLV-1感染者の割合が高い地域は，九州，沖縄，四国や紀伊半島の海岸線地域などであるが，東北や北陸の一部の海岸線地域，北海道の一部の地域でも割合が高いことが報告されている[2]。HTLV-1感染者の地域偏在の原因についてはよくわかっていない。民族移動などの人類学的歴史的背景が考えられている。HTLV-1は3つの遺伝子型，コスモポリタン型，中央アフリカ型，メラネシア型に分けられるが[3]，日本のHTLV-1はコスモポリタン型に属する。コスモポリタン型はさらにA~Eの5つのサブタイプが知られており，日本のHTLV-1はAとBである[4]。HTLV-1の遺伝子型による関連疾患の発症頻度の違いは報告されていない。

世界全体のHTLV-1感染者数は1000～2000万人と推定されているが，推定根拠が不明で正確な数は明ら

帝京大学大学院・公衆衛生学研究科
Graduate School of Public Health, Teikyo University
〒173-8605 東京都板橋区加賀2-11-1 TEL 03-3964-1211

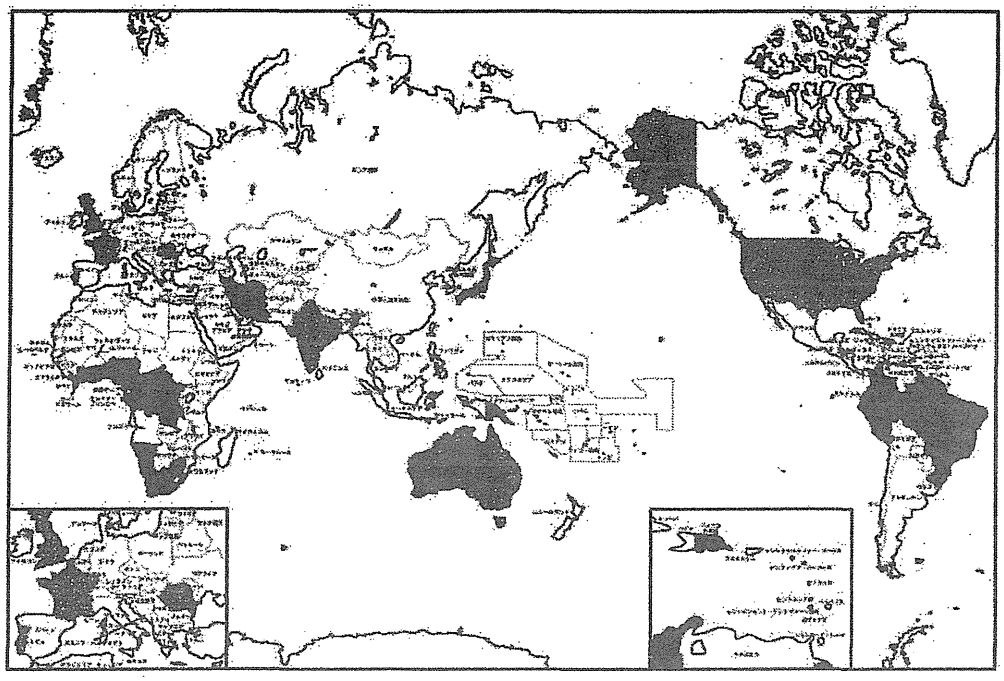

図1　HTLV-1感染者の世界分布
文献[1]より引用改変。黒塗りはHTLV-1感染者率1-5%，灰色は1%以下を示す。

かではない。日本全体のHTLV-1感染者数把握は，厚生労働省班研究として全国の初回献血者における地域別・年齢別HTLV-1抗体陽性率の結果と人口動態統計資料をもとに1988年と2008年に実施された。1988年の報告では，1988年単年の献血者の抗体陽性率を用いて日本全体のHTLV-1感染者数は約120万人と推定されたが[2]，HTLV-1感染者のほとんどが九州，沖縄，に集中しており，かつ若年者の陽性率が極めて低いことより，日本におけるHTLV-1感染者数は将来自然減するものと予測されていた。しかし，2006-2007年の献血者の抗体陽性率を用いた2008年の報告では，日本全体のHTLV-1感染者数は約108万人と推定され[5]，この20年間日本におけるHTLV-1感染者数はほとんど減少しておらず，1988年と比べ2008年では，九州・沖縄の感染者数は減少し，大都市圏の感染者数が増加していたことが判明している（図2）[5]。

一般にHTLV-1感染の大半は新生児期の母乳によって感染し，出生年時代の母乳哺育方法や家族構成の違いが影響し，HTLV-1感染率は年齢が高い（出生年が早い）ほど高い。また成人期以降は，女性の感染率が男性より上回るため，夫婦感染ルートによる感染率増加が示唆されている。ちなみにATL発症率は男性が高く，HAM/TSP発症率は女性が高い。

2．ATLの発症数と死亡数

ATL患者の地域分布はHTLV-1感染者の分布とほぼ等しいため割愛する。日本全体のATLによる年間死亡実数は，厚生労働省人口動態統計によると，1999年から2010年まで毎年ほぼ一定で約1000人である。しかし日本全体のATL発症実数は，人口動態統計では報告されていない。全国T・Bリンパ系腫瘍研究グループは日本全体のATL発症実数を把握するために，1988年から1999年まで基幹病院を対象としたATL全国実態調査を行い，日本におけるATLの年発症数は約700例と推定した[6]。しかし2010年に実施された厚生労働省班研究の報告では，年発症者数は約1100人と推定されている[7]。両者の発症数の違いは研究手法の違いによると思われ，日本全体の年間発症数は年間死亡数より1-2割ほど多いと推測されるが，正確な発症数は不明である。ATLの診断時年齢の平均値は，1990年調査時58.3歳，1999年調査時61.1歳，2009年調査時66.0歳としだいに高齢化していることが判明している[7]。

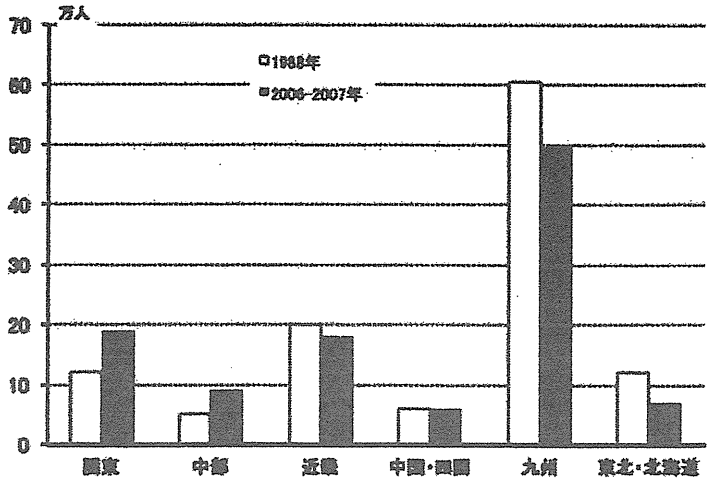

図2　日本におけるHTLV-1感染者数の推計値の地域別分布

文献[9]より引用改変。白カラムは1988年当時のHTLV-1感染者推計数を，黒カラムは2008年当時のHTLV-1感染者推計数を示す。

地域がん登録を行っている一部の県や市では，より詳細なATLの発症実数と人口10万人あたりの発症率が毎年報告されている。HTLV-1キャリアが多い長崎県がん登録の報告では，2000-2007年のATLの発症実数は，毎年男性51～65人，女性30～62人，2007年の県内粗罹患率は10万人あたり男性9.1，女性6.8であり，男性の発症率は同年の白血病罹患率（男性9.7，女性3.9）とほぼ肩を並べている。

3．HTLV-1キャリアからのATL発症率とリスクファクター

四国宇和島市（人口290,464, HTLV-1感染率；男5.4%，女8.3%）の調査研究では，30歳以上のHTLV-1キャリア10万人あたりのATL発症率は男性145，女性55.2，HTLV-1キャリアの生涯ATL発症率は男性6.9%，女性3.0%と推計されている[9]。長崎県の某諸島(人口26,870人，HTLV-1感染率；男14.3%，女17.9%）の調査研究では，30歳以上のHTLV-1キャリア10万人あたりのATL発症率は男性137.7，女性57.4，HTLV-1キャリアの生涯ATL発症率は男性6.6%，女性2.1%と推計され[9]，宇和島の結果とほぼ同じである。その他の報告も含め，HTLV-1キャリア1,000人あたり年間ATL発症率は男性1～1.5，女性0.5～0.7，30歳以上のHTLV-1キャリアにおける生涯ATL発症率は男性4～7%，女性2～5%と推定されている。　このようにATLは一部のHTLV-1キャリアからしか発症せず，大半のキャリアは無症状で過ごし「無症候性HTLV-1キャリア」と呼ばれている。

ATL発症にかかわるリスクファクターとしてこれまで報告されてきたさまざまの因子を表1にまとめる。母子間感染，男性，加齢，特定のHLA保持者，免疫の状態などの宿主側の要因や，感染細胞数の多さを反映する白血球増加，異型リンパ球増加，ウイルス抗体価上昇，可溶性IL-2R上昇などの臨床検査値の異常や，HTLV-1プロウイルス量の上昇などが報告されている。特にHTLV-1プロウイルス量はダイレクトに感染細胞数を反映していると考えられ，HTLV-1プロウイルス量の増加はキャリア状態からATL進展への重要なマーカーと考えられている[10]。しかし，HAM/TSPへ進展するキャリア症例でもHTLV-1プロウイルス量は高く，HTLV-1プロウイルス量の増加がATL進展にかかわる役割については未解明な部分が残っている。HTLV-1キャリア実数は女性が多いが，ATL発症率は男性が高く，一方HAM/TSP発症率は女性が高いこと，プロウイルス量が高値の無症候性キャリアが存在すること，免疫抑制剤投与中のHTLV-1キャリアから高率にATLが発症したという報告があることなどから，キャリアの免疫状態がATL発症を制御している可能性が示唆されている。

4．今後の課題

ATLは予後不良の血液悪性腫瘍であることから，発症予防や早期治療という戦略のためには，キャリアの中でATLを発症するリスクファクターを明確にし，発症のハイリスクグループを同定することが重要な課題である。しかし，どのようなHTLV-1キャリアが発症しやすいか，あるいは宿主側から見たATL発症のリスクファクターは何か

表　日本人 HTLV-1 キャリアから ATL 発症に関わるリスクファクターのまとめ

宿主の感受性	
	乳児期の母子感染
	50 歳以上の到達年齢
	男性
	HLA-A*26, HLA-B*4002, HLA-B*4006, HLA-B*4801
	糞線虫（*Strongyloides stercoralis*）感染
	免疫力低下
ウイルス感染量	
	HTLV-1 プロウイルス量の増加（100 PBMCs 中 4 コピー以上）
臨床検査マーカー	
	sIL-2R の上昇（500 U/mL 以上）
	抗 HTLV-1 抗体 titer の上昇（1,024 倍以上）
	末梢血異常リンパ球の増加（0.6% 以上）
	抗 Tax 活性低下
	白血球数の増加（9,000/μL 以上）
	単クローン性感染細胞の増加
	細胞表面マーカー発現の異常（CD26 発現の低下）

ATL = adult T-cell leukemia = 成人 T 細胞性白血病
HTLV-1 = human T-cell leukaemia virus type 1 = ヒト T 細胞性白血病ウイルス
HLA = human leukocyte antigen = ヒト白血球抗原
PBMC = peripheral blood mononuclear cell = 末梢血単核球細胞
sIL-2R = soluble interleukin-2 receptor = 可溶性インターロイキン 2 レセプター
さまざまの文献からの拾い上げ

ということについては全容解明にはいたっていない。遺伝的背景，免疫学的背景，分子生物学的特徴などを含む HTLV-1 キャリアの大規模コホート調査などを行い，ATL 発症のリスクファクター解明についてさらなるエビデンスを積み重ねる必要がある。

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Detection of Antibodies to Human T-Cell Leukemia Virus Types 1 and 2 in Breast Milk from East Asian Women

Futoshi Matsubara,*[,a] Yasuko Sagara,[b] Yoshihisa Kato,[c] Kouji Harada,[d] Akio Koizumi,[d] and Koichi Haraguchi[e]

[a] *Department of Microbiology and Biochemistry, Daiichi University of Pharmacy;* [e] *Department of Analytical Chemistry, Daiichi University of Pharmacy: 22–1 Tamagawa-cho, Minami-ku, Fukuoka 815–8511, Japan:* [b] *Department of Quality, Japanese Red Cross Kyushu Block Blood Center: 1–2–1 Kamikoga, Chikushino, Fukuoka 818–8588, Japan:* [c] *Department of Pharmacokinetics and Pharmacodynamics, Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University: 1314–1 Shido, Sanuki, Kagawa 769–2193, Japan: and* [d] *Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine; Yoshida, Sakyo-ku, Kyoto 606–8501, Japan.*

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We investigated the incidence of human T-cell leukemia virus type I (HTLV-1) infection in a total of 17 regions in four countries, including 13 regions in Japan, as well as Korea (Seoul and Busan), China, and Vietnam, by testing breast milk using a particle agglutination assay (PA) and line immunoassay (LIA). Among 266 samples from Japan, 24 (9.0%) were positive on PA and 3 (1.1%) were positive on LIA. Among 50 samples from Seoul, 2 were positive on PA and 1 was positive on LIA. In contrast, all 50 samples from Busan were negative on both tests, suggesting the maldistribution of HTLV-1 infectants in South Korea. The numbers of positive samples were 2/91 on PA and 1/91 on LIA for China and 1/88 on both PA and LIA for Vietnam. In China, one sample with a high probability of HTLV-2 infection was identified by LIA and synthetic peptide enzyme-linked immunosorbent assay (ELISA). We examined HTLV-1 antibody in breast milk samples using commercially available test kits, suggesting the existence of HTLV-1 carriers in endemic areas in Southeast Asia and an HTLV-2 infectant in China. As a part of human ethno-epidemiological research, these results constitute valuable epidemiological data. Further studies on the sensitivity, specificity, and reliability of assays using antibodies to HTLV-1 and 2 in breast milk will be necessary for large-scale epidemiological surveys of HTLV infection.

Key words breast milk; human T-cell leukemia virus type II; line immunoassay; particle agglutination assay

Adult T-cell leukemia (ATL) is a malignant CD4-positive T-cell neoplasm caused by infection with human T-cell leukemia virus type I (HTLV-1). Because HTLV-1 is a retrovirus, its genomic RNA is incorporated into target cells, and DNA is incorporated into the host genome as a provirus *via* reverse transcriptase.[1,2]

The prevalence of ATL is 0.2–0.3%, and a route of infection that poses a particular issue is vertical transmission (mother–child transmission) *via* breast milk.[2,3] ATL is endemic in equatorial Africa, the Caribbean islands, Colombia, Brazil, south India, Papua New Guinea, northeast Australia, and among indigenous people living at the margins of the Andes plateau in South America; in Japan, the infection rate is highest in western Japan, particularly Kyushu.[3–5]

In addition to blood transfusions and sexual intercourse, HTLV-1 infection can be transmitted from mother to child *via* breast milk, with the cause of vertical transmission being infection *via* lymphocytes in breast milk.[1–3] Pregnant women who are carriers must switch to infant formula in order to prevent infection. HTLV-1 testing is required for all pregnant women in Japan, with the main test methods used comprising antigen or antibody testing of blood.[4] Recently, ATL provirus DNA testing using automated nucleic acid purifiers has also been investigated.[6] In developing countries, however, blood testing may not always be adequate.

HTLV-2 has also been isolated from individuals other than leukemia patients, and its association with disease remains unclear. Although HTLV-2 carriers have been reported to be common in Central and South America, the regional distribution of HTLV-2 has not been investigated in detail.[7]

In our preceding paper,[8] we reported for the first time that the gelatin particle agglutination (PA) method can be used to measure HTLV-1 antibody in breast milk, which can be sampled non-invasively and is the major source of infection. This PA method is comparatively easy to use as needed, in both the laboratory and the field. For confirmatory testing, HTLV-1 was examined using the Innogenetics™ Inno-lia™ HTLV I/II Score. A combination of PA and line immunoassay (LIA) was used on breast milk.

The purpose of this study was to develop a screening assay for the detection of antibodies to HTLV-1/2 in breast milk from East Asian women and to further examine HTLV-1 and HTLV-2 incidence using the Innogenetics™ Inno-lia™ HTLV I/II Score and synthetic peptide enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Specimens A total of 545 breast milk samples (Beijing, 91; Hanoi, 88; Seoul, 50; Busan, 50; Okinawa, 33; Nagasaki 28, Yamaguchi, 20; Okayama, 20; Kochi, 10; Hyogo, 20; Wakayama, 15; Kyoto, 20; Fukui, 20; Gifu, 20; Tokyo, 20; Miyagi, 20; and Hokkaido, 20) were collected between 2004 and 2010, and were archived in the Kyoto University Human Specimen Bank. Written informed consent was obtained from all participants. The bank project was reviewed[9] and approved

The authors declare no conflict of interest.

* To whom correspondence should be addressed. e-mail: matsubara@daiichi-cps.ac.jp

by the Ethics Committee of the Kyoto University Graduate School of Medicine on 14 November 2003 (E25).

PA Screening tests were performed using 545 breast milk samples and a commercially available SERODIA HTLV-1 test kit (Fujirebio Inc., Tokyo, Japan) for *in vitro* diagnosis.[10] The kit includes HTLV-1 antigen-coated gelatin particles that agglutinate in the presence of HTLV-1 antibody in human serum or plasma. Test samples (25 μL) and positive control serum were prepared by 2-fold dilution up to 1:512. After an equal volume of sensitized particles was added, reactions were visually interpreted in duplicate. The agglutination patterns were interpreted according to the following criteria for an antibody titer of 1/8: negative (−), particles concentrated in the shape of a button with a smooth round outer margin; inconclusive (±), particles concentrated in the shape of a compact ring with a smooth round outer margin; positive (+), peripheral agglutination of the particles in a definite large ring with a rough multiform outer margin; and strongly positive (++), a film of agglutinated particles spread out uniformly on the bottom of the well.

LIA PA-positive breast milk was assayed for the presence of HTLV-1 antibodies using INNO-LIA™ HTLV I/II Score assays (Innogenetics N.V., Gent, Belgium), which were originally designed for testing serum or plasma. Milk samples (100 μL) were incubated in troughs containing LIA strips at 25°C overnight for 16 h. This incubation was followed by three washing steps with washing buffer before the addition of an alkaline phosphatase anti-human immunoglobulin conjugate. Samples were then incubated for 30 min at 25°C. Three washing steps were again performed, followed by incubation with the chromogen 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium for 30 min at 25°C. The results were compared to a positive control.

Synthetic Peptide-ELISA (sp-ELISA) LIA-positive breast milk was assayed by sp-ELISA[11] using three synthesized peptides (corresponding to the Gag p19 protein 100 to 130 aa, the Env gp46 protein 175 to 199 aa, and the Env gp46 288 to 317 aa) derived from HTLV-1 genome sequence ATK-1. The peptide corresponding to the region of the Env gp46 protein 175 to 199 aa (gp46-175) allowed the discrimination of HTLV-1/2 antibodies.

RESULTS AND DISCUSSION

We carried out preliminary screening of HTLV-1 antibody using the PA method with a total of 545 samples: 266 from 13 locations in Japan, and 279 samples from China, Vietnam, and South Korea (Seoul and Busan). Of these, 29 samples showed positive or inconclusive patterns of agglutination. LIA of these 29 samples revealed that three were indeterminate for HTLV-1 infection, four were inconclusive, and two were positive. Of the two positive samples, one from China also indicated positivity for HTLV-2, which is an extremely interesting result. Table 1 shows the overall results for PA and LIA. In the agglutination reaction, 516 of the 545 (94.7%) breast milk samples were negative. Confirmatory testing was required for 29 samples (5.3%), comprising 11 in which particles formed a compact ring with a smooth outer margin (±), eight in which

Table 1. Screening Assay for Antibodies to HTLV-1 in Breast Milk Samples Using PA and LIA

Country	Sampling area	Number of samples	Positive on PA	Positive on LIA
Japan	Okinawa	33	7	0
	Nagasaki	28	3	0
	Yamaguchi	20	0	0
	Okayama	20	0	0
	Kochi	10	0	0
	Hyogo	20	3	0
	Wakayama	15	0	0
	Kyoto	20	0	0
	Fukui	20	0	0
	Gifu	20	0	0
	Tokyo	20	0	0
	Miyagi	20	3	2
	Hokkaido	20	8	1
Japan total		266	24	3
China	Beijing	91	2 ±[a] ±[b]	1 −[a] ±[b]
Korea	Seoul	50	2 +[c] ±[d]	1 +[c] −[d]
	Busan	50	0	0
Vietnam	Hanoi	88	1 ±[e]	1 ±[e]
Total		545	29	6

Japanese data were reported by Matsubara *et al.*[8] Year of collection and age of sample donors were as follows: *a*) Sep. 2008, 27; *b*) Feb. 2009, 26; *c*) Jan. 2010, 31; *d*) Feb. 2010, 31; *e*) Sep. 2008, 19.

there was peripheral agglutination of the particles in a clear large ring with a rough multiform outer margin (+), and 10 in which a film of agglutinated particles was spread out uniformly on the bottom of the well (++). Table 1 also shows the donor locations of these 29 breast milk samples: seven from Okinawa, three from Nagasaki, three from Hyogo, three from Miyagi, and eight from Hokkaido were positive. Samples from all other Japanese regions were negative. Five samples (1.8%) from the other countries required confirmation, with two from Beijing, one from Hanoi, and two from Seoul being positive.

HTLV-1 and HTLV-2 were examined using the Innogenetics™ Inno-lia™ HTLV I/II Score using 10-fold quantities of samples according to the manufacturer's standard protocol. As shown in Table 1, two samples were positive and four had a deferred pattern. As shown in Fig. 1, a positive pattern for HTLV-2 was observed in one sample from China, and the sample did not react to gp46-175, suggesting that the donor may be an HTLV-2 carrier. Although it is difficult to distinguish between HTLV-1 and HTLV-2 using the PA method, Berini *et al.*[12] reported that the PA sensitivity of HTLV-2 in the blood of carriers is equal to that of HTLV-1.

The incidence of HTLV-1 infection is high in western Japan, particularly in Kyushu,[3–5,13] with some cases also found in Hokkaido.[14] and it has also been reported in equatorial Africa, the Caribbean islands, Colombia, Brazil, south India, Papua New Guinea, northeast Australia, and among indigenous people living at the margins of the Andes plateau in South America, which represent endemic areas of the virus.[2] As shown Table 1, with the exception of Japan, HTLV-1-endemic areas are often in developing countries, where blood testing may not be feasible. The PA method uses a freeze-dried product that is prepared when required, and determination is made by diluting the sample and visually observing agglutination images; thus, it can easily be used for preliminary screening and is effective for use in developing countries.

It seems that the PA positivity ratio in breast milk is higher than that in the blood, but the results of the LIA method in the present study were equivalent to those obtained with blood samples. Thus, although the PA method using breast milk is useful as a first screen, follow-up testing is necessary.

The sp-ELISA method uses ELISA-coated synthetic peptides corresponding to the immunodominant regions of HTLV-1 structural proteins,[11] and for this study we used p19 gag protein (100 to 130 aa), gp46 protein (175 to 199 aa), and gp46 (288 to 317 aa). The peptide corresponding to the region of the Env gp46 protein, 175 to 199 aa (gp46-175), allows the discrimination of HTLV-1/2 antibodies.

HTLV-2 has been isolated from individuals other than leukemia patients, and its association with disease remains unclear. It has also been found in locations including Central and South America,[7] Sweden,[15] Spain,[16] and Brazil,[17] and its roots are believed to lie in the indigenous peoples of areas such as the Florida peninsula of the United States, the Yucatan peninsula of Mexico, and Panama.[5] In this study, it was interesting that we detected an HTLV-2-positive infectant in China.

In countries such as Japan, the United States, France, and the Netherlands, blood donations are routinely checked for a variety of infectious diseases. Recently, China[18] and Korea[19] have reported epidemiological analyses of HTLV-1 and -2 infection, but in other Asian countries, screening has only been established in the last few years, if at all.[20] High-performance antibody screening, such as that on donated blood, is required to assess the status of HTLV infection and to prevent further infection. While it is useful to demonstrate the validity of screening breast milk as opposed to blood, it is important not only to facilitate simpler screening but also highly accurate screening. Further studies on the sensitivity, specificity, and reliability of assays using antibodies to HTLV-1 and -2 in breast milk are necessary for large-scale epidemiological surveys of HTLV infection.

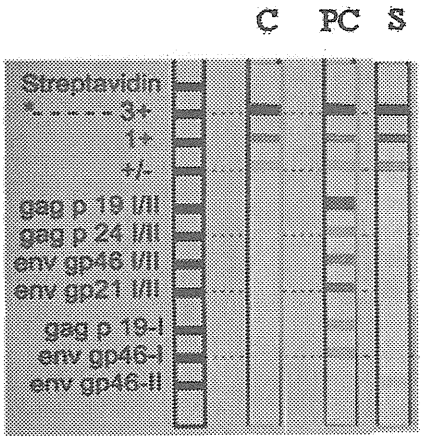

Fig. 1. Positive LIA Reactions of Milk Samples

C, control; PC, positive control; S, milk sample (HTLV-2 (±)).

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