ルスに関する基礎的な研究の発展はめざましいものがある。また，母子感染予防法の開発は大きな貢献といえる。しかし本邦においてはいまだ100万人以上のキャリアが存在しており，キャリアからウイルスを駆逐する方法や関連疾患を予防する方法は発見されていない。また今回の特集の主題「ストップザ性感染症」の観点からは男女間のHTLV-1の新規感染予防法については手つかずの状態であり，残された課題は大きい。我が国はいわゆる先進工業国の中では唯一のHTLV-1高浸淫国であり，これらの課題解決に今後も取り組んでいくことは世界的に対する貢献としてもぜひ必要であると思われる。

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HTLV-1感染症：母子感染予防対策とその課題

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1. はじめに

ヒトTリンパ向性ウイルス1型（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感染者（ウイルスキャリア）は世界的には本邦およびアフリカ，中南米を中心に見られる[2]．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おいて高頻度に見られることが示された[3]．この調査の時点では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[4]．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（図1）．

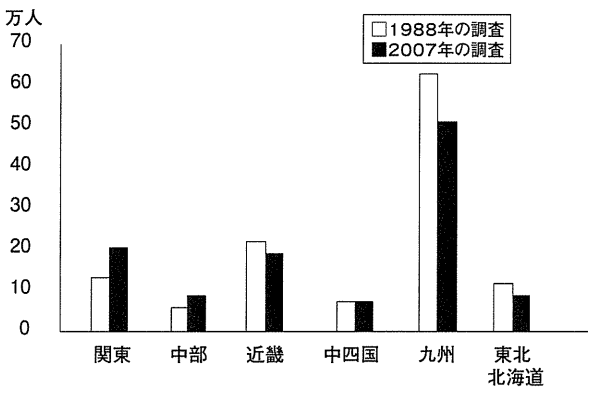

図1　地域別キャリア数の推移
（本邦におけるHTLV-1感染及び関連疾患の実態調査と総合対策．厚生労働科学研究費補助金研究事業研究班．平成21年度総括研究報告書より改変）

HTLV-1によって引き起こされる主な疾患は成人T細胞性白血病（ATL）やHTLV-1関連脊髄症（HAM），HTLV-1関連ぶどう膜炎である[5-7]．このほかにも皮膚疾患，肺疾患，膠原病の一部についても関連が示唆されている．ATLの発症頻度はHTLV-1キャリアにおいて年間1,000人に1名程度，生涯発症率は5％程度と推定されており，HAMの頻度はこれよりも低いと考えられている[8]．いいかえればHTLV-1キャリアの95％は生涯無症候性である．しかしながら特にATLは抗がん剤の多剤併用療法に対して抵抗性であり，予後の改善が期待できる骨髄移植を用いた治療の適応となる患者数も高齢発症の傾向があるため多くない．このため現在においてもATLは極めて予後不良な疾患である[9]．HTLV-1感染を予防することによりATLの発症を防ごうとする取り組みがキャリアの多い地域を中心に長年にわたり行われてきた[10]．本稿においてはHTLV-1母子感染予防についてのこれまでの取り組みと今後の展望・課題を紹介する．

2. HTLV-1の感染経路（表1）

HTLV-1キャリアにおいてウイルスは主にTリンパ球のゲノムに組み込まれたプロウイルス

Prevention of mother to child infection of HTLV-1

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として存在しており，ウイルス粒子を体液中より直接検出することは難しい．このため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―細胞間接触が起こることが必要である[11,12]．このような機会は自然界では母子間，性行為等に限られており，ウイルスの感染は基本的には家族内に限定される[13-15]．新規感染者は抗体が陽転し，生涯ウイルスが消失することはない．以前は存在した輸血を介した感染は1986年に赤十字血液センターにおいて抗体スクリーニングが行われるようになってからはなくなったと考えられている[12]．

ATLの発症予防という観点からは母子間感染の予防が重要である．これはHAMやぶどう膜炎が性行為や輸血等による感染後にも発症するのに対して，ATLはこれまでの疫学的なデータから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である[14,15]．さらに母子間で乳児期に感染したキャリアは配偶者間で感染したキャリアよりも高いウイルス量を示すことが判明しており，ATL発症のリスクと関連がある可能性がある[16]．しかしながら成人の性行為感染由来のHTLV-1キャリアからATLが発症しないというエビデンスはない．

HTLV-1の母子感染経路としては，当初子宮内感染，分娩時の産道感染，母乳を介した感染の3つが主に考えられた．その後，キャリアの母乳中に感染細胞が存在すること，その感染細胞を経口的に投与することによってマーモセットに感染が成立すること，キャリア母親から出生した児における感染率は母乳栄養児で高いことなどの研究成績により，HTLV-1の母子感染の主な経路は母乳を介したものであることが判明した[17-19]．

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
3．輸血による感染	1986年以降なし

3. 長崎県におけるHTLV-1母子感染予防の取り組み（表2）

以上のような成績をもとに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プロジェクトが1987年より長崎県において発足した．医師会，日本母性保護医協会（産婦人科医会），小児科医会，長崎大学，国立長崎中央病院，長崎県で構成される「長崎県ATLウイルス母子感染防止研究協力事業連絡会」が設置され，長崎県全域の産婦人科においてHTLV-1母子感染予防の取り組みが開始された．すなわち妊婦や関連の医療機関を対象にHTLV-1感染症および母子感染についての啓発活動が推進され，妊婦の承諾を得たのちに抗体スクリーニングが行われた．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[10]．キャリア母親から出生した児の感染率は，6ヶ月以上の母乳栄養で20.5%であったのに対して，6ヶ月未満の母乳栄養で8.3%，人工栄養で2.4%と有意に低下したことが示された．1998年にはインフォームドコンセントとカウンセリングを強化したプログラムが開始されたが，このプログラムでは母乳栄養を希望する母親には3ヶ月以内の短期母乳が推奨された．その結果，1999年～2006年の集計対象となった834人のキャリア母親において，71%が人工栄養，12.7%が短期母乳，13.8%が母乳栄養を選択した．フォローアップできた児の感染率は人工栄養3.2%，短期母乳3.0%，母乳栄養が15.8%であったとされている．

1987年から2007年までの20年間に長崎県においては約7,000人の母親がHTLV-1陽性である

表2　HTLV-1母子感染対策（栄養法による介入）と児の感染率

	児の感染率
1．人工栄養	2～3%
2．3カ月未満の母乳栄養	3～7%
3．凍結母乳による栄養	不明

ことが判明した．人工栄養を選択した母親の割合を80％とした場合，児の感染はこのプロジェクトにより20.3％から2.5％に低下したと考えられた．この結果約1,000人の児のキャリア化が阻止され，そのことにより50人程度の将来のATL 発症を予防したと推測されている．

4. HTLV-1総合対策

妊婦における HTLV-1抗体スクリーニングは九州沖縄以外の地域においても大多数の産科施設で行われていることが2009年のアンケートにより示された[20]．また献血者を対象とするHTLV-1感染率の全国調査により2008年の時点で全国に約108万人の HTLV-1キャリアが存在すると推定された．このような実態をもとに2010年９月に内閣総理大臣の指示により「HTLV-1特命チーム」が設置され，12月に「HTLV-1総合対策」が取りまとめられた．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，母子感染予防に関しては全国の妊婦健診の標準的な項目に HTLV-1抗体検査が追加され公費負担の対象となった[21]．さらに HTLV-1母子感染予防のための保健指導，カウンセリング充実のための研修会の実施，マニュアルの作成等が開始されている．

5. HTLV-1感染症対策の課題

① HTLV-1母子感染予防の全国展開にともなう啓蒙・情報提供・カウンセリング

長崎県において素晴らしい成果をあげた HTLV-1母子感染予防対策であるが，この事業を成し遂げるためには，研究者のみならず関連する産科小児科の医療従事者や自治体，保健指導関係者の多大な努力が必要であったと思われる．全国的に同様の対策を行う場合，特に HTLV-1キャリアの頻度が少ない地域においてはさまざまな問題が発生しうる．妊娠中という重要な時期に初めて HTLV-1感染を知る妊婦の不安やショックの大きさは容易に推測できる．しかしこれらの地域においては HTLV-1感染症と関連疾患について知識の豊富な医療従事者や保健指導関係者の絶対数が少ない．このためキャリア母親のカウンセリングを行うことが容易でない．さらに ATL 患者の発症頻度が非常に少ない地域では，その診療にかかわった経験のある医療関係者も少ないため，その疾患を予防する重要性についての医療関係者の認識が得られにくいことが危惧される．このような点から「HTLV-1総合対策」にもとづいて進められている HTLV-1母子感染予防のための保健指導，カウンセリング充実のための研修会の実施，マニュアルの作成等を充実させる必要がある[21]．

② HTLV-1抗体測定法：判定保留の問題

HTLV-1キャリアはすべて抗体が陽性であり，抗体検査によりキャリアと同定されてきた．抗体のスクリーニング検査は多くの場合粒子凝集法や化学発光法等が行われ，スクリーニング検査陽性例について確認検査として免疫ブロット法が主に用いられている．しかしながらスクリーニング検査陽性検体が確認検査で陰性や判定保留となるケースがみられ，とくに HTLV-1キャリアの頻度が少ない地域においてはスクリーニング陽性検体の多数がこのような結果を示すことが問題となっている[20]．研究班の報告によれば，長崎県においては妊婦スクリーニング検査 HTLV-1抗体陽性と判定された8,504検体は確認検査により85.4％が陽性，14.6％が陰性と判定され判定保留はなかったが，東京都においてはスクリーニング検査陽性と判定された36検体は確認検査により25％が陽性，55.6％が陰性となり，判定保留が19.4％あった．母子感染予防の観点からは，特に免疫ブロット法で判定保留となった妊婦に人工栄養を勧めるか否かということについては一定の見解がない．判定保留の場合に血清学的な方法ではなく，末梢血液細胞より HTLV-1プロウイルスを検出する PCR 法を用いる検査が検討されている．しかしいまだ標準的で汎用性にある検査としては確立されていない現状である．

③ 母乳以外の感染経路

HTLV-1キャリア母親から出産後人工栄養で育てた児においても２％程度抗体が陽転化しキャリアとなる場合があることが判明している．このような児の感染経路としては子宮内感染，産道における感染，それ以外の感染

(唾液そのほか)が可能性として挙げられる．臍帯血液細胞についてHTLV-1プロウイルスの有無を検討した結果，PCR陽性例は検出されたがその後の児のキャリア化とは関係なかったことが示されている[22]．このため子宮内感染についてはその存在を完全には否定できないものの，人工栄養児のHTLV-1陽性化の原因としてその関与は少ないと考えられており，産道感染がより疑われている．しかし，キャリア化率およびその後のATL発症率の低さを考慮に入れて，現在のところ，産道感染予防のための帝王切開等は勧められていない[23]．人工栄養児におけるHTLV-1キャリア化は少数であるが，授乳をあきらめたにもかかわらず児にHTLV-1感染が成立した場合の母親の心情を考えれば，この経路の解明も重要な課題である．

④　母乳遮断以外の方法による新規感染防止策

現在ほかに有効な方法がないために行われている人工栄養によるHTLV-1母子感染予防策であるが，本来自然な栄養法である授乳による児の栄養が望ましいことはいうまでもない．３ヶ月以下の短期母乳栄養では感染率が下がることは判明しているが，人工栄養に比較すると感染率は高いとされており[23]，また３ヶ月目の時点で断乳することの困難さも指摘されている．短期母乳以外の方法として，凍結保存することによって感染力を喪失させた母乳を与える方法も知られている．しかし現実的には長期間これを行うことは母親の多大な労力を要する．今後の可能性のひとつとしてＢ型肝炎ウイルスキャリア母親からの出産で行われているようなワクチンによる母子感染予防法の開発がある．これは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．しかしながら現在のところHTLV-1感染に対するワクチン開発はまったくの基礎研究レベルにとどまっている．さらに通常の母乳栄養を行った場合なぜ一部（おおよそ20%）の児にのみHTLV-1の母子感染が成立し，そのほかの80%の児には起こらないかということについても理由は判明していない．新規感染のハイリスク因子が判明すれば，人工栄養を勧めないといけない妊婦を絞り込むことができる可能性がある．

⑤　キャリアからのATL発症予防法の開発

新たな感染を予防することは重要であるが，現在すでに国内に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．HTLV-1キャリアの95%は生涯HTLV-1関連疾患とは無縁である．このためどのようなキャリアが関連疾患を発症し，どのような方法でそれを阻止することができるか解明することが重要である．最近の研究によりウイルス量が高いキャリアからATLが発症することが明らかとなってきた[24,25]．配偶者間で感染が成立したキャリアのウイルス量の比較からは，ウイルス量はウイルスの性質ではなくそれ以外の因子で決まることが判明している[26]．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関連疾患の防止に大きく貢献できる可能性がある．

6. ま と め

HTLV-1母子感染予防事業では母乳による栄養法の中止を勧めることになるが，それは重篤で予後の悪い疾患であるATL発症を予防できるという観点から許されると考えられている．この事業は大変な労力を要するが，HTLV-1感染とそれに関連する疾患を撲滅することが可能であることを示すことは，世界の中でも多数の感染者をかかえる唯一の先進工業国である日本の責務と思われる．また新規感染を遮断すると同時に，現在国内に108万人存在するとされるHTLV-1キャリアからのATLやHAMのような関連疾患発症を予防する方策を開発することも同じように重要である．

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Leukemia (2012) 1 - 9

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ORIGINAL ARTICLE

Clinical significance of CADM1/TSLC1/IgSF4 expression in adult T-cell leukemia/lymphoma

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Cell adhesion molecule 1 (CADM1/TSLC1) was recently identified as a novel cell surface marker for adult T-cell leukemia/lymphoma (ATLL). In this study, we developed various antibodies as diagnostic tools to identify CADM1-positive ATLL leukemia cells. In flow cytometric analysis, the percentages of $CD4^+CADM1^+$ double-positive cells correlated well with both the percentages of $CD4^+CD25^+$ cells and with abnormal lymphocytes in the peripheral blood of patients with various types of ATLL. Moreover, the degree of $CD4^+CADM1^+$ cells over 1% significantly correlated with the copy number of the human T-lymphotropic virus type 1 (HTLV-1) provirus in the peripheral blood of HTLV-1 carriers and ATLL patients. We also identified a soluble form of CADM1 in the peripheral blood of ATLL patients, and the expression levels of this form were correlated with the levels of soluble interleukin 2 receptor alpha. Moreover, lymphomas derived from ATLL were strongly and specifically stained with a CADM1 antibody. Thus, detection of $CD4^+CADM1^+$ cells in the peripheral blood, measurement of serum levels of soluble CADM1 and immunohistochemical detection of CADM1 in lymphomas would be a useful set of markers for disease progression in ATLL and may aid in both the early diagnosis and measurement of treatment efficacy for ATLL.

Leukemia advance online publication, 6 January 2012; doi:10.1038/leu.2011.379

Keywords: CADM1/IgSF4/TSLC1; ATLL

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATLL) results from infection with human T-lymphotropic virus type 1 (HTLV-1).[1,2] Following HTLV-1 infection, 2.1 to 6.6% of HTLV-1 carriers will develop ATLL, and most of the ATLL patients will die within a year.[3] An estimated 10–20 million people worldwide are infected with HTLV-1, and HTLV-1 is endemic in southwestern Japan, the island of Kyushu, Africa, the Caribbean Islands and South America.[4] ATLL cells are mainly derived from activated helper T cells with the $CD3^+$, $CD4^+$, $CD8^-$ and $CD25^+$ (also known as interleukin 2 receptor alpha (IL-2Rα)) cell surface markers.[2] A fraction of ATLL cases have been shown to also express forkhead box P3 (*FOXP3*), which is a master gene for regulatory T cells (T-reg), suggesting that some cases of ATLL may originate from HTLV-1-infected T-reg cells.[5,6] For diagnosis, identification of mono- or oligoclonal provirus integration events by Southern blot analysis is one of the definitive markers for ATLL. In addition to viral integration, ATLL cells with multi-lobulated nuclei (called 'flower cells') have been frequently seen in leukemia cells in the peripheral blood of ATLL patients. Hypercalcemia and high levels of either serum lactate dehydrogenase (LDH) or soluble IL-2Rα (sIL-2Rα) have been found to be unfavorable markers for ATLL; however, these markers are not specific for the diagnosis of ATLL.[7,8]

The developmental steps of ATLL after HTLV-1 infection have remained obscure for 30–40 years. HTLV-1 Tax is thought to be an important viral protein that functions in the maintenance of HTLV-1-infected lymphocytes;[9,10] however, expression of Tax protein was not detected in over 70% of ATLL cases because of genomic deletion and/or DNA methylation.[11–14] Recently, HTLV-1 basic leucine zipper (HBZ) was found to be constitutively expressed in ATLL cells and was shown to interact with JUN and CREB2 to regulate Tax expression.[15,16] HBZ also promotes $CD4^+$ T-cell proliferation in transgenic mice;[16] therefore, HBZ has important roles and functions not only in maintaining the virus life cycle but also in the maintenance of the HTLV-1-infected cells that contribute to disease pathogenesis. Although HBZ is expressed in the majority of ATLL cells, only 5% of HTLV-1 carriers develop ATLL, suggesting that additional factors besides viral infection are required for the development of ATLL.

To identify additional pathogenic factors or novel surface markers for ATLL, we collected gene expression profiles for acute-type ATLL. Using a comprehensive DNA microarray gene expression analysis, we recently demonstrated that cell adhesion molecule 1 (CADM1/TSLC1/IgSF4) is a novel cell surface maker for ATLL.[17] CADM1 was initially isolated as a tumor suppressor for lung cancers by genomic analysis. CADM1 expression is reduced in a variety of cancers by promoter methylation and is associated with poor prognosis and enhanced metastatic potential.[18] By contrast, we identified that high expression of CADM1 has an important role in enhanced cell–cell adhesion to the vascular endothelium, tumor growth and the organ infiltration of ATLL cells.[19]

In this study, we developed various antibodies for CADM1 to be used as diagnostic tools for identifying ATLL leukemia cells.

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Received 14 April 2011; revised 24 November 2011; accepted 29 November 2011

We successfully identified ATLL cells in the peripheral blood and in lymphoma samples and detected the soluble form of CADM1 in the peripheral blood of ATLL patients using specific antibodies for CADM1. The CADM1 antibody may therefore represent a useful tool in the diagnosis of ATLL cells.

MATERIALS AND METHODS

Quantification of HTLV-1 proviral load

HTLV-1 proviral DNA load was determined by real-time PCR as previously described.[20] Briefly, genomic DNA from peripheral blood mononuclear cells (PBMCs) was extracted by proteinase K digestion and phenol/chloroform extraction and then subjected to a real-time TaqMan PCR assay using an ABI PRISM 7000 detection system (Perkin Elmer/Applied Biosystems, Forster City, CA, USA) with two sets of primers specific for the *pX* region of the HTLV-1 provirus and the human gene encoding the RNase P enzyme. The primers and the probe for the *RNase P* gene were purchased from Applied Biosystems; those for the *pX* region of the HTLV-1 provirus were described previously.[20] Genomic DNA of normal control PBMCs mixed with a plasmid DNA, which contained almost the whole genome of the HTLV-1 provirus (*Sac*I site of 5′-LTR to *Sac*I site of 3′-LTR), was used as a standard to quantify the proviral DNA copies. The copy number of the plasmid DNA was calculated based on the size and weight of the plasmid DNA, as measured by spectrophotometry. HTLV-1 proviral loads in some of the PBMC samples were measured by the Group of Joint Study on Predisposing Factors of ATL Development (JSPFAD, Japan) as described previously.[21] The amount of HTLV-1 proviral DNA was calculated as the copy number of HTLV-1 per 100 PBMC = ((copy number of *pX*)/(copy number of *RNase P*/2)) × 100.

RESULTS

Frequent expression of surface CADM1/TSLC1 among ATLL-derived cell lines

CADM1/TSLC1/IgSF4 was identified as a novel surface marker on ATLL cells by gene expression profiling using DNA microarray analysis and was found to be frequently expressed in leukemia cells from patients with acute-type ATLL.[17] We first analyzed the CADM1 protein levels in a panel of T-leukemia cell lines using a chicken anti-human CADM1 antibody (MBL, Nagoya, Japan). A 107 kDa band was clearly detected in whole-cell lysates from the KOB, KK1 and S1T cell lines (Figure 1a), which have been reported

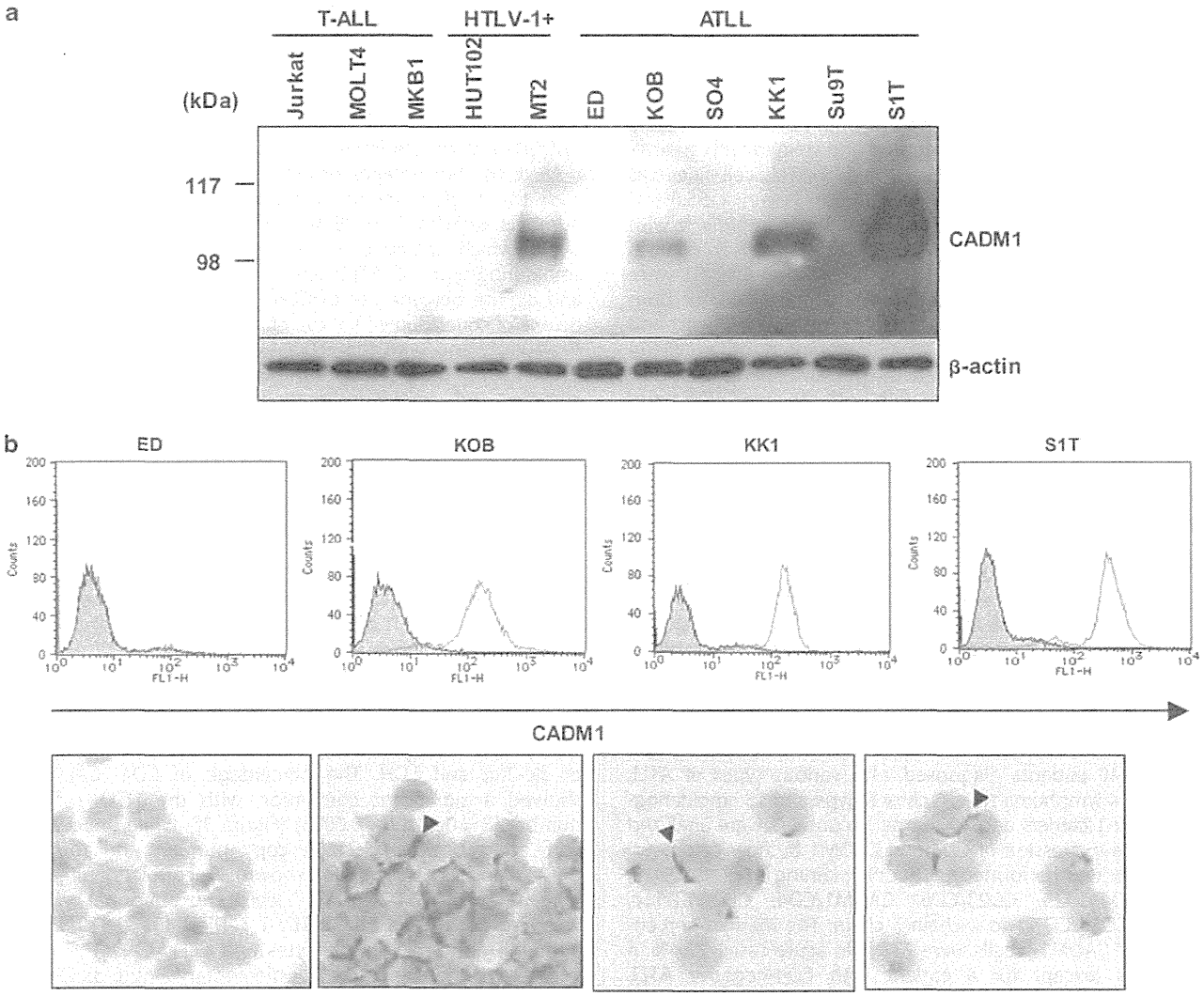

Figure 1. High CADM1 expression in ATLL analyzed by immunoblot, flow cytometry (FMC) and immunohistochemical staining (IHC). (**a**) Immunoblot analysis was performed on a series of T-lymphoid leukemia cell lines (three T-ALL, T-acute lymphoid leukemias; two HTLV-1+, HTLV-1-infected cell lines; six ATLL, ATLL-derived cell lines) with a chicken anti-human CADM1 antibody. (**b**) A human anti-human CADM1 antibody (051-054), which was established by phage display, was used for FMC and IHC. The anti-CADM1 antibody was visualized by Alexa 488 in FMC and by horseradish peroxidase in IHC.

to express CADM1 according to reverse transcriptase PCR and northern blot analysis.[17] To confirm CADM1 expression on the cell surface of ATLL cells, we examined CADM1 membrane expression by flow cytometry with an Alexa 488-labeled human anti-CADM1 antibody generated by phage-display technology.[22] Four ATLL cell lines were used for flow cytometry: CADM1-negative ED and CADM1-positive KOB, KK1 and S1T cell lines. In all three CADM1-positive cell lines, the fluorescence intensity of CADM1 expression was two logs greater than that of the isotype immunoglobulin G control (Figure 1b, upper panels), while only background levels of fluorescence could be seen in the CADM1-negative ED-ATLL cell line, which had high levels of DNA methylation in the CADM1 promoter region.[17] To evaluate the subcellular distribution of CADM1, immunohistochemical staining was performed on the same cell lines using the anti-CADM1 antibody (Figure 1b, bottom panels). CADM1 was highly concentrated at the cell–cell contact sites in the three CADM1-positive cell lines, and no staining of CADM1 was detected in the ED cell line. These data suggest that CADM1 expression in ATLL cells may promote cell-to-cell contact.

Low levels of CADM1 expression in the T-reg fraction of peripheral lymphocytes

To examine the expression of CADM1 in peripheral blood T-lymphocytes of healthy volunteers, T-reg populations were analyzed for CADM1 expression because $CD4^+CD25^{high}$ T-reg cells are a potential source of ATLL cells.[5,6] Initially, the $CD4^+CD25^+$ cell fraction was separated from PBMCs of a healthy volunteer by the magnetic bead method and stained with an anti-CADM1 antibody. Almost 100% of the S1T-ATLL cell line was strongly stained with the anti-CADM1 antibody; however, 55.8% of the $CD4^+CD25^+$ cells were stained weakly in comparison with the high level of staining of S1T-ATLL cells (Figure 2a). To confirm whether the purified $CD4^+CD25^+$ cells expressing CADM1 were T-reg cells, the sorted $CD4^+CD25^+$ cells were stained for both FoxP3 (a master regulator in the development of T-reg cells) and CADM1. In all, 93% of the $CD4^+CD25^+$ double-positive cells in the peripheral blood were stained by the anti-FoxP3 antibody, while 37% of the cells were stained with both the anti-CADM1 and anti-FoxP3 antibodies (Figure 2b), suggesting that a fraction of the $CD4^+CD25^+FoxP3^+$ T-reg cells weakly expressed CADM1 on their cell surfaces.

We then determined the proportion of $CD4^+CADM1^+$ and $CD4^+CD25^+$ T cells in PBMCs from 10 healthy volunteers after selection with Cy5-labeled CD45 staining. On average, 7.3% of $CD45^+$ cells in PBMCs expressed CD4 and CD25, while only 0.6% of the cell population expressed CD4 and CADM1 (Figure 2c and representative fluorescence-activated cell sorting data are shown in Supplementary Figures 1a and b), indicating that the number of $CD4^+CADM1^+$ cells was significantly lower than the number of $CD4^+CD25^+$ cells in the PBMCs of healthy volunteers. To determine the percentage of $CD4^+CADM1^+$ cells in peripheral lymphocytes of various types of ATLL and HTLV-1 carriers, $CD45^+$ PBMCs from 40 patients diagnosed with various types of ATLL (7 acute-type, 4 lymphoma-type, 6 chronic-type and 23 smoldering-type), 51 HTLV-1 carriers and 10 normal volunteers were analyzed for the surface expression of CD4 and CADM1 by flow cytometry analysis, which was performed by double staining of CD12/CD19, CD3/CD8, CD4/CD25, CD23/CD5, CADM1/CD4, CD20/CD11c, CD16/CD56, CD30/CD7 and κ-chain/λ-chain. The median percentages of $CD4^+CADM1^+$ cells were 73.9% in acute cases, 72.4% in chronic cases (except for a patient with CD4-negative ATLL described below), 5.6% in lymphoma cases, 11.5% in smoldering cases, 4.4% in HTLV-1 carriers and 0.5% in normal volunteers (Figure 2d). In these subjects, the percentages of $CD4^+CD25^+$ cells were significantly correlated with those of $CD4^+CADM1^+$ cells ($R=0.907$, $P<0.0001$) (Figure 2e), suggesting that most of the ATLL cells were $CD4^+CD25^+CADM^+$. However, we also observed a cell surface profile of $CD3^+CD8^-$ (91.3%), $CD25^+CD4^-$ (81.5%) and $CD4^-CADM1^+$ (83.6%) in a case of chronic ATLL, suggesting that the surface markers of the ATLL cells represented $CD4^-CD8^-$ double-negative T lymphocytes that expressed CD25 and CADM1.

CADM1 expression in leukemia cells from ATLL patients and HTLV-1-infected cells from HTLV-1 carriers

To confirm that most of the HTLV-1-infected ATLL cells were indeed in the $CD4^+CADM1^+$ cell fraction, PBMCs from an HTLV-1 carrier and two ATLL patients with chronic or smoldering ATLL were isolated and separated into CADM1-positive and CADM1-negative cell fractions by anti-CADM1 antibody-conjugated magnetic beads. The cell fractions were then analyzed for the expression of CD4 and CADM1 by fluorescence-activated cell sorting analysis (Supplementary Figure 2). In these three patients, 3.4 to 31.4% of PBMCs were positive for CD4 and CADM1. After separation by the magnetic CADM1 antibody, 73.5 to 96.5% of the cells were $CD4^+CADM1^+$. To assess whether these $CD4^+CADM1^+$ cells indeed represented the HTLV-1-infected cell population, the HTLV-1 status was determined by PCR of the proviral DNA with primers against the *HBZ* region of the HTLV-1 genome. As shown in Figure 3a, the HTLV-1 genomic sequence was detected in the three CADM1-positive cell fractions, while weak or no signal was detected in the CADM1-negative cell fractions, indicating that the majority of HTLV-1-positive cells are present in the CADM1-positive cell fractions.

Next, the percentages of $CD4^+CADM1^+$ cells were compared with those of abnormal lymphocytes or with the DNA copy numbers of HTLV-1 in PBMCs of patients with various types of ATLL, which included 6 acute-type, 8 chronic-type and 6 smoldering-type of ATLL, and 20 HTLV-1 carriers (Figures 3b and c). The percentages of $CD4^+CADM1^+$ cells showed a high degree of correlation with those of abnormal lymphocytes ($R=0.791$, $P<0.0001$) and with the HTLV-1 DNA copy numbers ($R=0.677$, $P<0.0001$) in these patient samples. Notably, in two samples from chronic- and smoldering-type ATLL patients, the number of $CD4^+CADM1^+$ cells was less than one-half of the number of HTLV-1 DNA copies (32.0% vs 107.97 copies and 30.0% vs 65.76 copies), which may be due to multiple copies of proviral DNA in the cells. In addition, the percentages of $CD4^+CADM1^+$ cells were correlated with the levels of sIL-2Rα ($R=0.586$, $P<0.0001$) and with the levels of LDH ($R=0.486$, $P=0.0015$) (Figures 3d and e). Consistent with earlier studies, both serum sIL-2Rα and LDH levels were correlated with the HTLV-1 DNA copy numbers ($R=0.705$; $P<0.0001$ and $R=0.44$; $P=0.0045$, respectively) in this study (data not shown).

To further evaluate the diagnostic efficacy of measuring CADM1-positive cells to detect HTLV-1-infected cells, the copy number of the HTLV-1 provirus in PBMCs of carriers was compared with the percentages of $CD4^+CADM1^+$ cells and the serum levels of sIL-2Rα and LDH. The percentage of $CD4^+CADM1^+$ cells showed a significant correlation with the HTLV-1 DNA copy number ($R=0.921$, $P<0.0001$) (Figure 3f), while there was a poor correlation between HTLV-1 copy number and the levels of sIL-2Rα and LDH (data not shown). A correlation between the percentage of $CD4^+CADM1^+$ cells and abnormal lymphocytes was also observed in the HTLV-1 carriers ($R=0.819$, $P<0.0001$), although abnormal lymphocytes and $CD4^+CADM1^+$ cells were very rare in these subjects (Supplementary Figure 3). On the basis of these data, in addition to the determination of copy numbers of HTLV-1 proviral DNA, quantification of $CD4^+CADM1^+$ cell number by flow cytometry may be useful for monitoring the number of HTLV-1-infected cells in the peripheral blood of ATLL patients and HTLV-1 carriers.

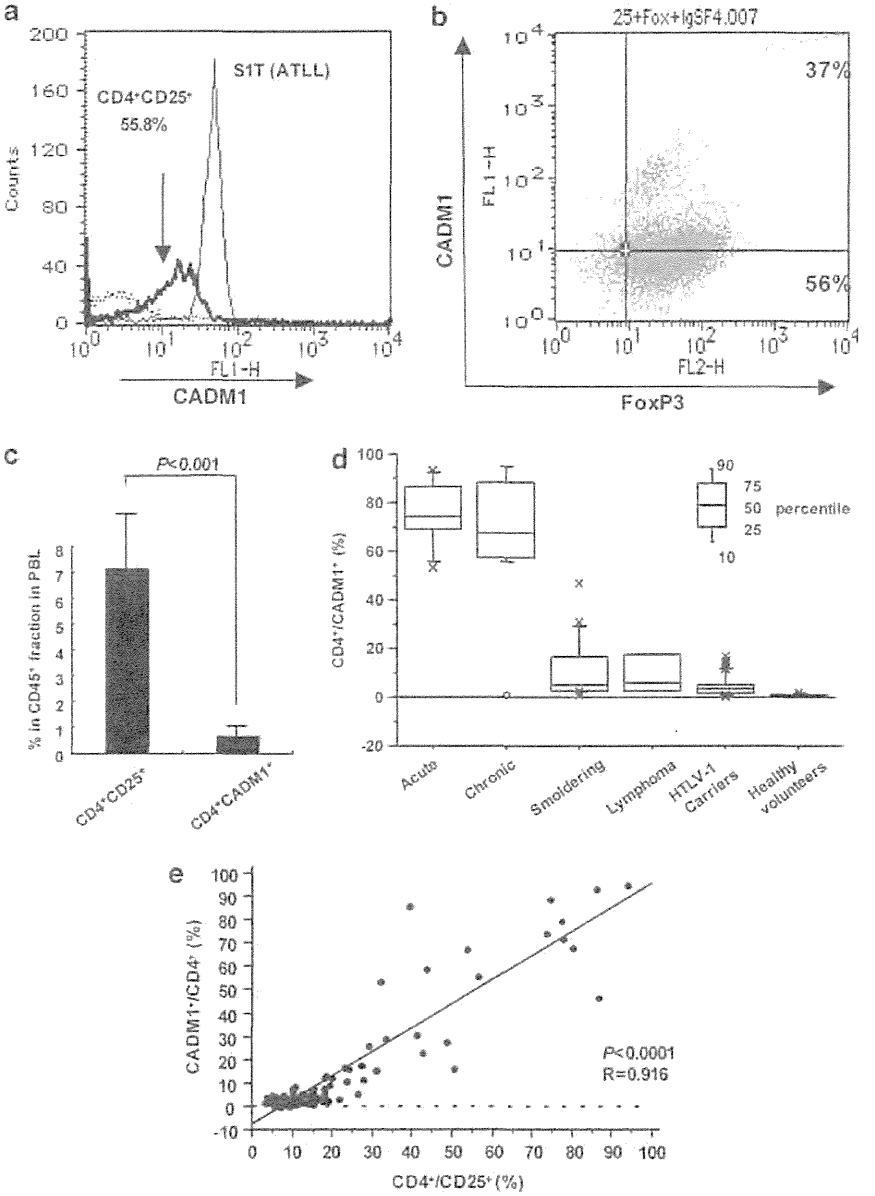

Figure 2. Flow cytometric analysis of CADM1 in T-reg lymphocytes, ATLL cells and HTLV-1-infected T cells. (**a**) Flow cytometric analysis of CADM1 expression in the $CD4^+CD25^+$ fraction from peripheral T lymphocytes. Each sample was stained with an Alexa 488-labeled anti-CADM1 antibody. The S1T-ATLL cell line with high CADM1 expression was used as a positive control. (**b**) The $CD4^+CD25^+$ fraction from peripheral lymphocytes was stained by the Alexa 488-labeled anti-CADM1 and PE-labeled anti-FoxP3 antibodies. (**c**) Comparison of percentages between the $CD4^+CD25^+$ and $CD4^+CADM1^+$ cell fractions in the $CD45^+$ fraction of peripheral blood lymphocytes. (**d**) Box plots are shown for the percentages of the $CD4^+CADM1^+$ cell fractions in $CD45^+$ peripheral blood lymphocytes from patients with various types of ATLL, HTLV-1 carriers and healthy volunteers. The data from a CD4-negative ATLL case are indicated by a white circle. (**e**) Comparison between $CD4^+CADM1^+$ and $CD4^+CD25^+$ cell fractions in $CD45^+$ peripheral blood lymphocytes from patients with various types of ATLL, HTLV-1 carriers and healthy volunteers. Spearman correlation coefficients were calculated to assess the association between $CD4^+CADM1^+$ and $CD4^+CD25^+$ cell fractions.

The soluble form of CADM1 is detected in the serum of ATLL patients

A soluble isoform of CADM1 consisting of the extracellular domain was recently isolated in murine mast cells.[23] We determined whether the soluble form of CADM1 was present in the serum of ATLL patients by western blot using a chicken anti-human CADM1 antibody. As a positive control, soluble CADM1 was produced by transfection of 293 cells with a construct encoding a soluble form of CADM1 (1 to 374 aa). The soluble CADM1 band (72 kDa) and the recombinant soluble form of CADM1 were clearly detected in the sera of five patients with acute-type ATLL but not in the sera of five healthy volunteers (Figure 4a). We screened the sera of 5 healthy controls and 25 ATLL patients (14 acute-type, 7 lymphoma-type, 2 smoldering-type and 2 HTLV-1 carrier) for the presence of soluble CADM1. We detected different levels of soluble CADM1 among these ATLL patients by western blot (data not shown). In addition, we compared the levels of soluble CADM1 in the serum and the percentages of $CD4^+CADM1^+$ cells in the peripheral blood (Supplementary Figure 4) and confirmed that high levels of soluble CADM1 are present in the serum of patients who had high numbers of $CADM1^+$ cells in the peripheral blood. As serum levels of soluble IL-2Rα are correlated with the prognosis

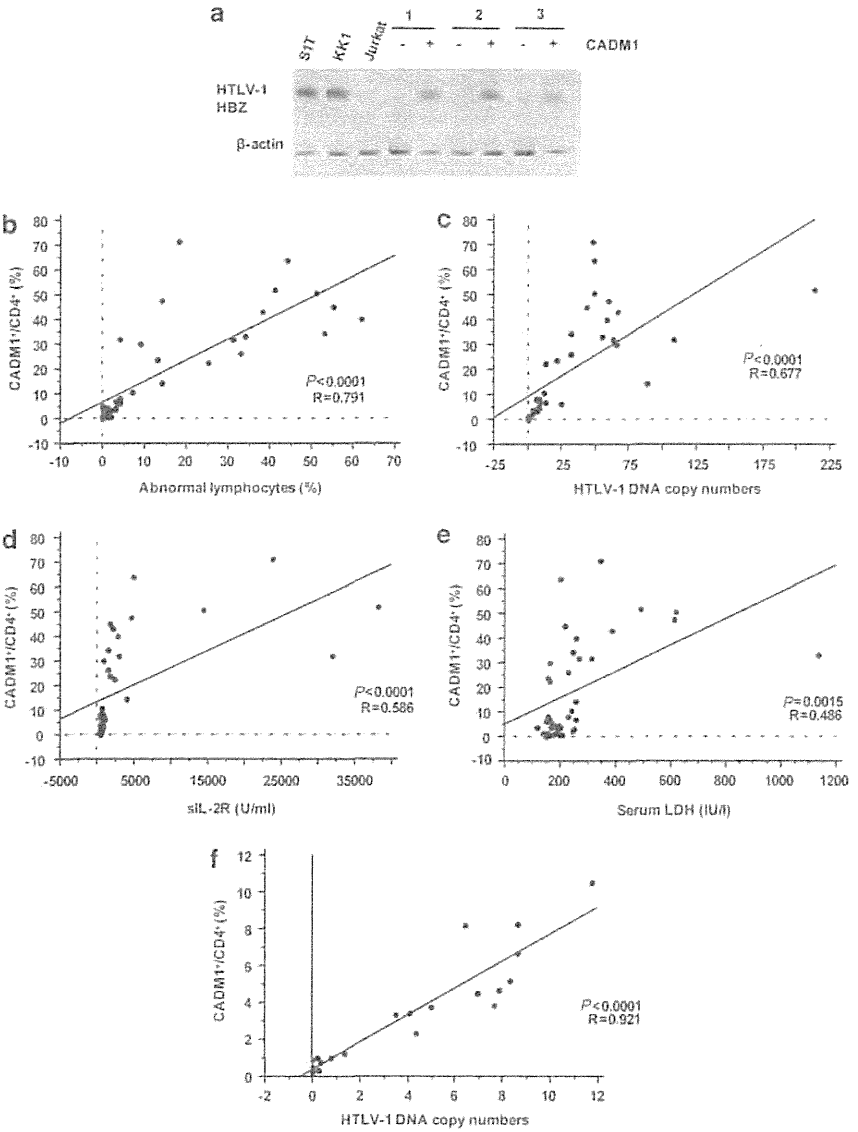

Figure 3. Correlation of the percentages of the $CD4^+CADM1^+$ fraction with the percentages of abnormal lymphocytes, HTLV-1 DNA copy number and the levels of soluble IL-2Rα and serum LDH in both various types of patients with ATLL and HTLV-1 carriers. (**a**) Identification of the HTLV-1 genome by PCR amplification after separation by CADM1-magnetic beads. After separation of the peripheral blood of three ATLL patients by magnetic beads, genomic DNA was extracted from both the CADM1 and non-CADM1 fractions and amplified by specific PCR primers for HTLV-1 *HBZ*. Two ATLL cell lines (S1T and KK1) were used as positive controls, and a T-ALL cell line (Jurkat) was used as a negative control for the HTLV-1 *HBZ*. Lane 1, smoldering ATLL; lane 2, chronic ATLL; lane 3, HTLV-1 carrier. (**b-e**) The percentage of the $CD4^+CADM1^+$ fraction was compared with the percentage of abnormal lymphocytes, the HTLV-1 DNA copy number and the levels of soluble IL-2Rα and serum LDH in both various types of patients with ATLL and HTLV-1 carriers. In (**d**), data from one acute-type patient were not included in the analysis because of the extremely high levels of soluble IL-2Ra ($CD4^+CADM1^+$, 32.9%; IL-2Ra, 96 900 U/ml). (**f**) The percentage of the $CD4^+CADM1^+$ fraction was compared with the HTLV-1 DNA copy number in HTLV-1 carriers.

of ATLL patients, we compared the serum levels of soluble CADM1 and soluble IL-2Rα in individual cases. As shown in Figure 4b, significantly higher levels of soluble CADM1 were detected in the serum of ATLL patients who had increased levels of soluble IL-2Rα; thus, serum CADM1 levels may be a diagnostic tool for the prediction of disease progression in ATLL.

High expression of CADM1 in ATLL-derived lymphomas

To examine the expression of CADM1 in tissue sections from lymphoma-type ATLL, formalin-fixed lymphoma samples from different types of malignant lymphomas were immunostained with the anti-CADM1 antibody. For these studies, we used a monoclonal antibody (1-10C) raised against the recombinant extracellular domain of the CADM1 protein. To confirm the reactivity of the anti-CADM1 antibody in formalin-fixed ATLL cells, cell pellets from various leukemia cell lines were fixed in 10% formalin, embedded in paraffin and stained for CADM1. The anti-CADM1 antibody specifically stained the surface of the CADM1-positive S1T-ATLL cell line but did not react with the CADM1-negative ED-ATLL and all non-ATLL cell lines (Figure 5a, panels 1 and 2, and Supplementary Figure 5a). Western blot analysis confirmed the lack of CADM1 expression in these cell lines (Figure 1a and Supplementary Figure 5b). We next performed immunostaining of lymph node biopsies from ATLL patients with malignant lymphoma using the anti-CADM1 antibody. As positive controls, we used erythrocytes and peripheral nerve tissue (Figure 5a, panels 3 and 4).[17,18] In addition, we examined CADM1

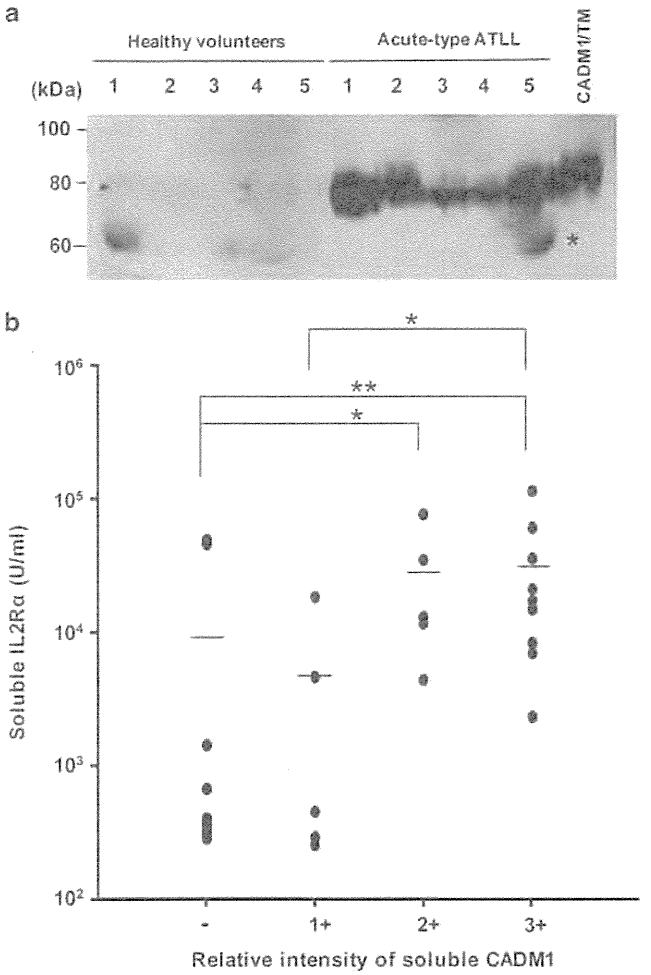

Figure 4. Identification of a soluble form of CADM1 in ATLL patients. (**a**) The soluble form of CADM1 in the peripheral blood from five healthy volunteers and five patients with acute-type ATLL was identified by immunoblot analysis using an anti-CADM1 antibody. The asterisk indicates an albumin band. Truncated CADM1 with an extracellular domain was purified from the culture supernatant of 293 cells after transfection of the CADM1 expression plasmid as a positive control. (**b**) The relative band intensity of CADM1 by immunoblot was compared with the level of sIL-2Rα in various serum samples from healthy volunteers, HTLV-1 carriers and ATLL patients. The band intensity was measured by the Image Gauge software (Fujifilm, Tokyo, Japan). The signal intensities were classified as either high (3+), medium (2+), low (1+) or undetectable (−). Asterisks indicate a significant difference between the band intensities of the groups (*$P<0.001$, **$P<0.0001$).

expression in three cases of lymph nodes with reactive follicular and/or paracortical hyperplasia (reactive lymph nodes) and found that most of the lymphocytes in the reactive lymph nodes were negatively stained and <1% of the cells were positively stained (Figure 5a, panel 5). The staining pattern of the CADM1-positive cells in the reactive lymph nodes mainly shows a uniform cytoplasmic pattern rather than the specific membranous staining that was seen in ATLL cells (as shown below and in Figure 1b). The CADM1-positive cells in reactive lymph node possibly correspond to histiocytes, including dendritic cells because a subset of T-cell zone dendritic cells was reported to express CADM1 (Necl-2) within the lymph node.[24,25] We examined 90 tissue samples from patients with various types of lymphoma, including 36 patients with ATLL and 54 with non-ATLL lymphomas, using erythrocytes and nerve fascicles as positive controls. Of the non-ATLL samples, 29 cases were T- or NK-cell lymphomas, 37 cases were B-cell lymphomas and 2 cases were null-cell lymphomas. Using a four-grade scale to score CADM1 immunohistochemical staining (0 to 3+, Figure 5b), we found that 92% of ATLL lymphomas were positive for CADM1, and 50% of them were heavily stained and were scored 2+ or higher (Table 1). Of note, a few lymphoma cells showed diffuse cytoplasmic staining in addition to membrane staining with CADM1. Among the non-ATLL lymphomas, a few CADM1-positive cells were observed, the morphology of which was small to medium in size with normochromatic round to ovoid nuclei and lacking nuclear atypia (Figure 5c). Based on the morphology and the CADM1-staining patterns, the CADM1-positive cells in the non-ATLL lymphomas were not considered as lymphoma cells but may correspond to histiocytes, including dendritic cells, because these cells were similar to the CADM1-positive cells found in reactive lymph nodes (Figure 5a, panel 5 and Figure 5c). Based on these results, a high degree of cell membrane staining for CADM1 with a score of 2+ may provide high specificity in the diagnosis of ATLL, and combined staining with CADM1 and other T-cell-specific markers may be necessary for a more accurate diagnosis of lymphoma-type ATLL.

DISCUSSION

In this study, we made a series of antibodies against CADM1 to be used as diagnostic tools for ATLL, such as for the identification and separation of ATLL and HTLV-1-infected cells, the detection of the soluble form of CADM1 in peripheral blood and the pathological identification of lymphoma-type ATLL after formalin fixation. Expression of CADM1 by flow cytometry was clearly detected on the surface of ATLL cells and HTLV-1-infected T lymphocytes, which was confirmed by detection of the HTLV-1 genome after separation by magnetic beads with a CADM1 antibody. The percentage of $CD4^+CADM1^+$ cells in the peripheral blood correlated highly with the DNA copy number of HTLV-1 in lymphocytes from HTLV-1 carriers and ATLL patients. In particular, we identified the soluble form of the CADM1 protein in the peripheral blood of HTLV-1 carriers and ATLL patients. The definitive diagnosis of ATLL is based on the confirmation of ATLL cells in the peripheral blood or in lymphoma tumors by detection of HTLV-1 genomic integration; therefore, measurement of serum levels of soluble CADM1 protein as well as detection of $CD4^+CADM1^+$ cells in the blood, when used in conjunction with other standard diagnostic methods, would be useful for identifying and monitoring disease progression in HTLV-1 carriers with increased accuracy and may aid in the early diagnosis and measurement of treatment effects for ATLL.

It has been proposed that HTLV-1 infects various types of cells, including T-reg cells and subsets of T helper cells (Th2 and Th17), in a cell-to-cell manner.[26–29] There is also evidence that ATLL cells act as T-reg cells that express CD4, CD25 and FoxP3 and are thought to contribute to the immune suppression of ATLL patients;[6] however, it was reported that CADM1 is expressed at low levels on resting naive T cells, and its expression is further downregulated 14 h following TCR activation.[30] Therefore, we determined the expression of CADM1 in the T-reg cell fraction of the peripheral blood of healthy volunteers. The results showed that a subset of the T-reg fraction weakly expressed CADM1, suggesting that CADM1 is not a major marker for the T-reg fraction and that CADM1 expression on ATL cells may reflect the fact that ATL cells originate from T-reg cells. As ATLL cells that constitutively express CD25 exhibited heterogeneous Foxp3 expression patterns,[5] a part of ATLL is likely derived from $FoxP3^+$ T-reg cells. In another report, a population of $FoxP3^+$ cells distinct from ATLL cells was shown to have a regulatory function and was found to impair the cell-mediated immune response to HTLV-1 in patients with ATLL.[31] Although we do not know whether the population of T-reg cells with weak expression of CADM1 in the

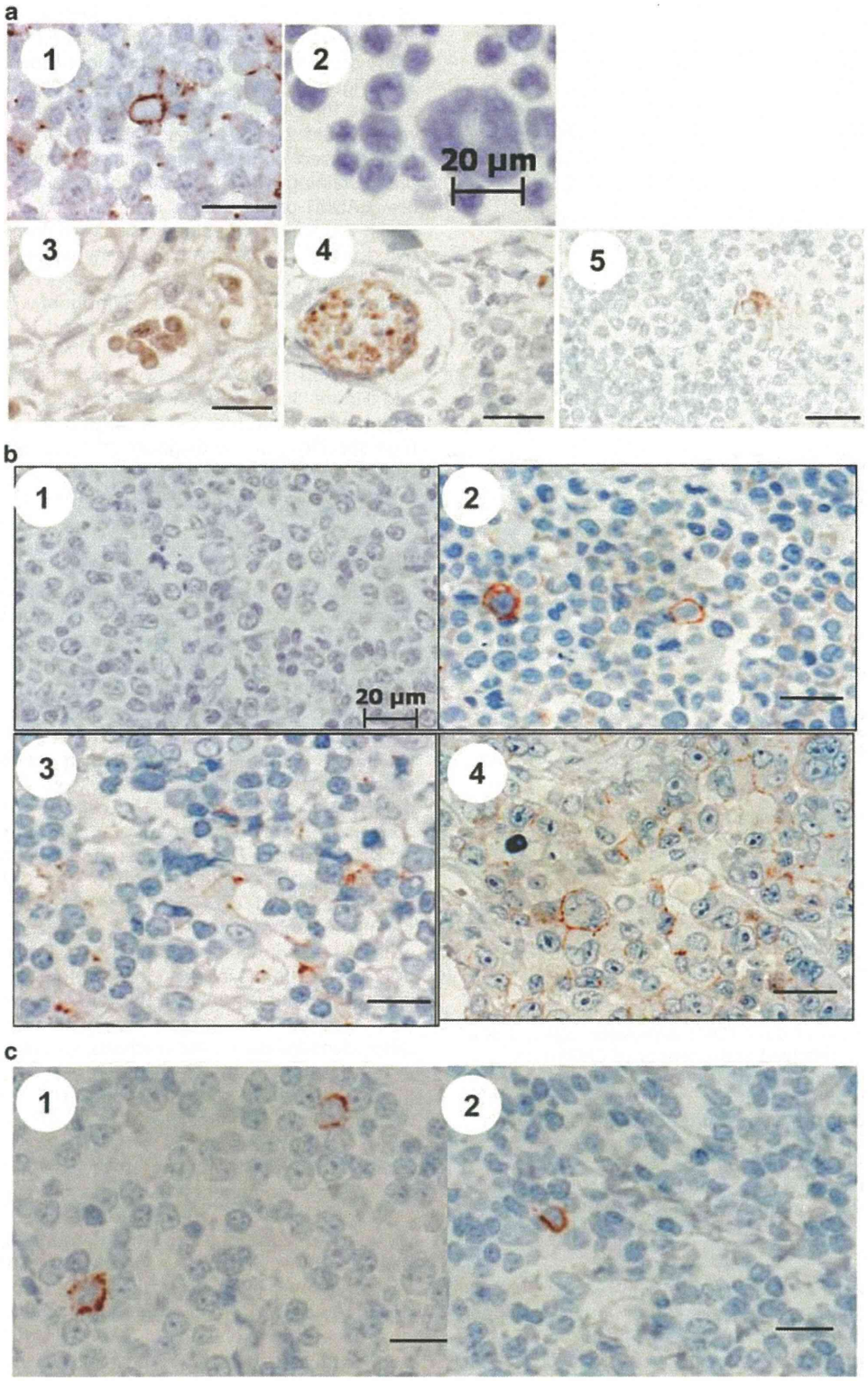

Figure 5. Expression of CADM1 in lymphoma-type ATLL. (**a**) Immunostaining of CADM1 in the S1T-ATLL cell line was used as a positive control (a1), and the ED-ATLL cell line was used as a negative control (a2) for CADM1 expression using an anti-CADM1 antibody (1–10C). Immunostaining of erythrocytes in the blood vessels (**c**), peripheral nerve cells (a3) and reactive lymph nodes (a4) using the same antibody. Scale bar, 20 μm. (**b**) The immunoreactivity for CADM1 was scored using a standardized four-tiered scoring scale as follows: staining in >30% of cells was scored as 3+ (b4); staining in >5% but <30% of cells was scored as 2+ (b3); staining in <5% of cells was scored as 1+ (b2); and a lack of staining was scored as 0 (b1). These images were taken from immunostained ATLL lymphoma sections. Scale bar, 20 μm. (**c**) Representative CADM1 immunostaining in B-cell (c1) and NK-cell (c2) lymphomas. Scale bar, 20 μm.

Table 1. Immunohistochemical staining of CADM1 in various types of lymphomas, including ATLL

	Case numbers	Staining scores				Positive rates (%)	
		Negative	1+	2+	3+	⩾1+	⩾2+
ATLL	36	3	15	14	4	33/36 (92)	18/36 (50)
Non-ATLL	54	37	16	1	0	17/54 (31)	1/54 (1.8)
T/NK	15	12	3	0	0	3/15 (20)	0/15 (0)
B	37	23	13	1	0	14/37 (38)	1/37 (2.7)
Null	2	2	0	0	0	0/2 (0)	0/2 (0)

Abbreviations: ATLL, adult T-cell leukemia/lymphoma; CADM1, cell adhesion molecule 1. The immunoreactivity for CADM1 was scored using a standardized four-tiered scoring scale as follows: staining in >30% of cells was scored as 3+; staining in >5% but <30% of cells was scored as 2+; staining in <5% of cells was scored as 1+; lack of staining was scored as 0.

PBMCs of healthy volunteers is the cellular origin for ATLL cells, CADM1 is thought to be one of the major markers for the various types of ATLL cells. In fact, we observed strong expression of CADM1 in rare cases of ATLL characterized by the $CD4^+CD8^+$, $CD4^-CD8^+$ or $CD4^-CD8^-$ phenotypes (data not shown); therefore, the $CADM1^{high}$ population of T-lymphocytes in peripheral blood can be considered ATLL cells.

The question of why CADM1 is strongly expressed on the surface of various types of ATLL remains unclear. Previously, we investigated whether the expression of CADM1 was induced by HTLV-1/Tax expression and found that Tax protein expression did not activate the expression of CADM1 in JPX-9 cells (data not shown). We also introduced a Tax expression vector into MOLT4 and 293T cells and determined the expression level of CADM1. We found that Tax could not induce CADM1 expression in these cells, suggesting that Tax expression is not related to the high expression of CADM1. As HBZ is known to be constitutively expressed in both HTLV-1-infected cells and ATLL cells and can modulate transcription of cellular genes,[16] it is possible that HBZ activates CADM1 expression. We also speculate that $CADM1^{high}$ expression in ATLL cells may be associated with transcriptional abnormalities in ATLL cells through the accumulation of genomic or epigenomic alterations. In this study, we found a good correlation between HTLV-1 copy numbers and the percentages of $CD4^+CADM1^+$ cells in the peripheral blood of HTLV-1 carriers, suggesting that HTLV-1 carriers with high percentages of $CD4^+CADM1^+$ cells could be associated with progressive genetic alterations and might be at high risk for developing ATLL.

Recent studies have shown that a few markers, such as CCR4 and CD70, are unique ATLL surface markers.[32,33] Although the proportion of $CD4^+CCR4^+$ cells and $CD4^+CD70^+$ cells in the PBMCs from healthy individuals were found to be approximately 5%,[27,33] the proportion of $CD4^+CADM1^+$ cells was <1% (Figure 2); therefore, measurement of $CADM1^+$ T cells is particularly efficient in the diagnosis of HTLV-1 infection in individuals who carry a small number of HTLV-1-infected cells. We have demonstrated previously that CADM1 has important functions in increasing cell adhesion and mediating progression to organ invasion.[19] In this study, we succeeded in isolating a low percentage of both HTLV-1-infected cells from the PBMCs of HTLV-1 carriers with high HTLV-1 copy numbers and ATLL cells from patients with ATLL. The sorted HTLV-1-infected cells and ATLL cells could become useful tools for transcriptional and/or genomic analysis that may be used to compare their results with those of PBMCs from either healthy volunteers or peripheral leukemia cells from patients with ATLL. The results may provide important information on the expression patterns and/or genomic abnormalities that occur at the early stages of HTLV-1 infection and/or ATLL development.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Grants-in-Aid for Scientific Research of Priority Area and for twenty-first Century COE program (Life science) from the Ministry of Education, Culture, Sports, Science and Technology, Japan Leukemia Research fund, and Research fund from Miyazaki Prefecture Collaboration of Regional Entities for the Advancement of Technological Excellence, JST.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

2．気道感染症
2）HTLV-1 関連気管支病変

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Keywords ● 成人 T 細胞白血病ウイルス 1 型，成人 T 細胞白血病，気管支病変，呼吸器病変発症病態，呼吸器病変画像所見／human T-cell leukemia virus type 1（HTLV-1），human T-cell leukemia（ATL），bronchial disorder, mechanism of respiratory disorder, images of respiratory disorder

要旨 ● HTLV-1 は，成人 T 細胞白血病などの原因ウイルスである。HTLV-1 関連炎症性疾患および HTLV-1 キャリアに HTLV-1 関連肺疾患を認めることが指摘されているが，その病態および発症機序，臨床所見などについては不明な点が多い。今回 HTLV-1 関連肺疾患の臨床像や画像所見の検討とともに，発症病態に関与する解析を行った。

1 はじめに

日本はレトロウイルスである成人 T 細胞白血病ウイルス 1 型（human T-cell leukemia virus type 1：HTLV-1）キャリアの多い国であり，約 110～120 万人いるといわれ，約半分が南西九州，沖縄に存在し，ほかは四国，紀伊，北陸，東北沿岸部にみられる。最近では東京・大阪などの大都市部で症例が増加している。

HTLV-1 ウイルス関連脊髄症やブドウ膜炎症例，無症候性キャリアで，肺病変を認めることが以前より指摘されているが，「HTLV-1 関連肺疾患」とは，成人 T 細胞白血病（adult T-cell leukemia：ATL）などを発症していない HTLV-1 キャリアに認められる肺病変を指す場合と，抗 HTLV-1 抗体陽性者にみられる肺病変すべてを含む概念など確実な定義は確立されていない。キャリアに認められる肺病変に関しては HTLV-1 associated bronchopneumonopathy（HAB）[1]，HTLV-1 associated bronchiolo-alveolar disorder（HABA）[2] という名称が提唱されている。その中で病変の画像所見には，小葉中心性の粒状影を示すびまん性汎細気管支炎（diffuse panbronchiolitis：DPB）パターン，慢性気管支炎パターンなどの「気管支病変タイプ」と，usual interstitial pneumonia（UIP）パターンなどの「間質性肺炎（interstitial pneumonia：IP）タイプ」などのさまざまな報告が散見される[3][4]。本稿では文献的考察を交えながら，主に「HTLV-1 関連気管支病変」を中心に述べていく。

2 HTLV-1 関連気管支病変に関する報告

HTLV-1 関連気管支病変は画像所見が類似の DPB と比較されることが多い。Kadota らはそれらを比較して（HTLV-1 陽性 15 例），初診時の年齢・性別・臨床症状・一般血液検査・呼吸機能検査・胸部 CT 所見に有意な相違点はなかったと報

HTLV-1 Associated Bronchiolo Disorder
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告した[5]。また気管支肺胞洗浄（bronchial alveolar lavage：BAL）液中の IL-2R 陽性細胞が HTLV-1 陽性患者で有意に高値であった。そして DPB に有効なマクロライド少量長期療法は，HTLV-1 関連気管支病変患者においては，15%ほど有用な症例もあるが，その他はほとんど無効であった。

Yamamoto らは HTLV-1 陽性（12 例）と陰性の DPB 症例を比較し，陽性者で閉塞性換気障害の程度が強く，上葉を侵す割合が高く，また BAL 中の $CD3^+/CD25^+$細胞と $CD8^+/CD25^+$細胞の割合が高く，サイトカイン・ケモカインの MIP-1α と IP-10 レベルが高かったと報告した[6]。迎らの HTLV-1 陽性（8 例）と陰性の DPB 症例の比較では，陽性例で BAL 中の $CD3^+/CD25^+$細胞が 2 例で著増，末梢血の $CD3^+/CD25^+$と $CD4/HLA\text{-}DR^+$細胞が増加していたが，組織学的な差異は認めなかったとの報告であった[7]。兼島らは HTLV-1 陽性と陰性者との比較で，慢性気道病変として DPB，慢性気管支炎（chronic bronchitis：CB），副鼻腔気管支症候群（sinobronchial syndrome：SBS）などをチェック，HTLV-1 陽性 DPB/陰性 DPB，陽性 CB/陰性 CB との間で比較している。結果として HTLV-1 陽性 CB において，副鼻腔炎の合併率が高い傾向があり，HTLV-1 陽性 DPB/CB において，末梢血のリンパ球幼若化試験で無刺激でも自己増殖を示している症例がみられたが，それ以外の項目においては両者間に大きな違いはなかったと報告している[8]。木村は DPB 様陰影の HABA 症例からはその後 ATL の発症が多く，IP 様陰影の HABA 症例からはその後肺癌の発症が多かったと報告している[3]。

3 HTLV-1 関連肺疾患の発症機序

HTLV-1 関連肺疾患の病態や発症機序については不明な点が多い。

HTLV-1 の Tax 遺伝子特異的な $CD8^+$ T リンパ球による細胞障害性の機序などにより，T リンパ球の肺胞炎やリンパ球性間質性肺炎などの肺病変や各種の臓器障害が起こることがいわれている[9]。また Tax 遺伝子による G1 期での T 細胞の細胞周期の停止，NF-κB の活性化による抗アポトーシス作用，p53 の抑制による DNA 損傷修復の阻害や[10]，主に $CD4^+$ リンパ球に感染した HTLV-1 が肺胞上皮に感染し，NF-κB，AP-1 を活性化することで発症するという報告もある[11]。また HTLV-1 に特徴的な pX 領域より生成される TAX 蛋白と HBZ 蛋白は細胞増殖と免疫応答において重要な役割を有している[12]。TAX は NF-κB，CREB/ATF，AP1 を活性化し T 細胞系増殖を促進することが知られており，これらを介して炎症性サイトカインの発現を促進している。一方 HBZ は無症候性キャリアの T 細胞にも発現し，プロウイルス量と相関が指摘されており，HTLV-1 関連脊髄症の重症度と HBZ の発現は相関も認められている。HBZ は免疫応答からの immune escape にも重要な役割を果たしており，TAX とともに HTLV-1 関連肺疾患の発症と病態に深く関与すると考えられている。

4 当科での HTLV-1 関連肺疾患に関する検討

1）BAL 液解析による検討

HTLV-1 関連 DPB 様症例における BAL 細胞における $p40^{tax}$（HTLV-1 の非構造蛋白）と各種炎症性サイトカイン，ケモカインの発現とその相関について検討した[13]〔HTLV-1 陽性 DPB 様症例群 10 例（男性 1 例，女性 9 例；年齢 60.1±10.1 歳。図 1 に症例を示す）。コントロール群 7 例（男性 4 例，女性 3 例；年齢 53.4±12.6 歳，抗体陰性，肺陰影なし）〕。HTLV-1 陽性群ではコントロール群と比べ，BALF 中の総細胞数とリンパ球の割合が有意に増加していた。HTLV-1 陽性群 10 例中 8 例で $p40^{tax}$の発現が認められ，その発現は BALF 中リンパ球比率と相関している傾向がみられた。またコントロール群に比べ，BALF 細胞中の IFN-γ，IL-2，MCP-1，MIP-1α，IP-10 mRNA の発現が有意に亢進していた。また BALF 細胞中の $p40^{tax}$の発現が IFN-γ，MIP-1α の発現と，IFN-γ と IP-10 の発現がリンパ球比率と有意に相関していた。これらのことから $p40^{tax}$の発現が局所の炎症性サイトカインやケモカイン産生に関与し，

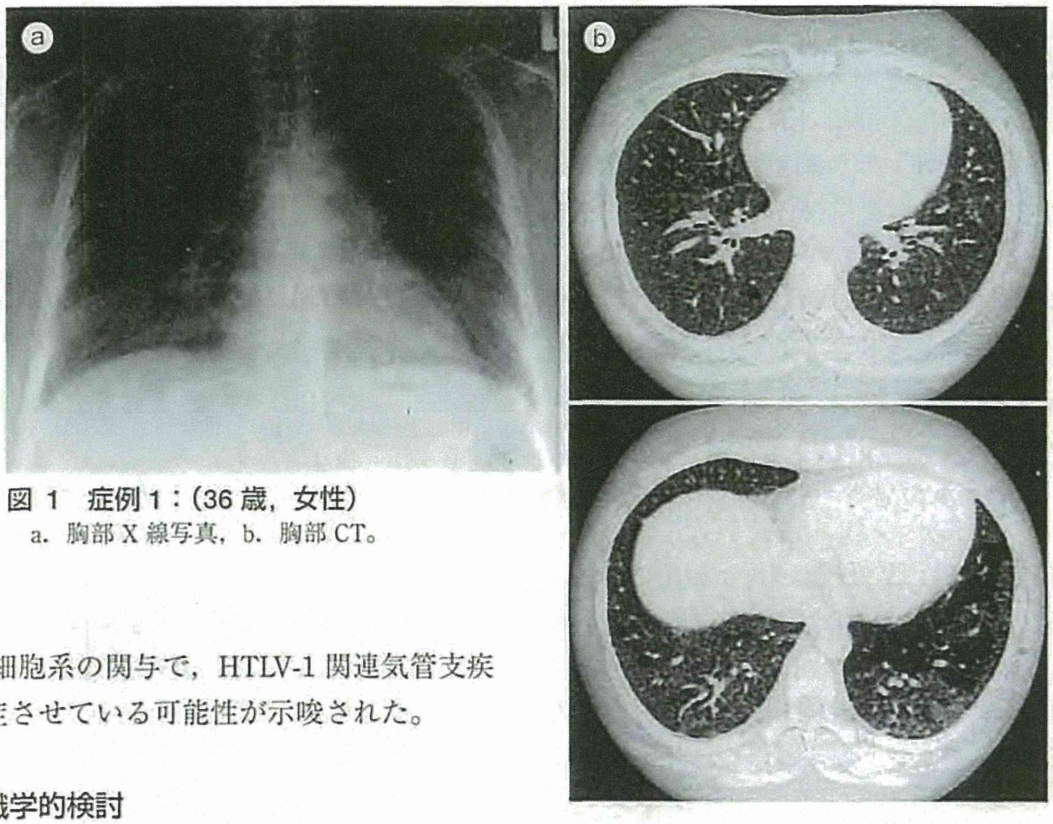

図 1　症例 1：(36 歳，女性)
a．胸部 X 線写真，b．胸部 CT。

特に Th1 細胞系の関与で，HTLV-1 関連気管支疾病変を発症させている可能性が示唆された。

2）肺組織学的検討

HTLV-1 関連肺疾患症例の肺組織における組織学的検討の結果，肺上皮細胞と浸潤しているリンパ球に TAX の発現を確認している[11]。

3）BAL 施行症例における抗 HTLV-1 抗体陽性者の肺病変に関しての画像的検討

a．方法

当科における約 12 年間の BAL 施行症例のべ 467 例のうち，抗 HTLV-1 抗体を測定し得た 228 例での検討。

b．結果

①抗 HTLV-1 抗体を測定し得た肺病変のある BAL 症例 228 例のうち，74 例 32.5%が抗 HTLV-1 抗体陽性であった（本邦の抗 HTLV-1 抗体陽性率の報告は地域によって 0.3 から 30%と差があるが，32.5%は高値）。平均年齢 60.7 歳。男性：女性＝24：50 人（そのうち ATL 発症は男性：女性＝5：13 人，HTLV-1 キャリアで DPB 様所見は男性：女性＝3：13 人，HTLV-1 キャリアで IP 様所見は男性：女性＝6：1 人と性差あり）。

②HTLV-1 抗体陽性 74 例の BAL 施行時の肺野陰影の内訳：ATL 発症 18 例（ニューモシスチス肺炎 5 例，DPB 様陰影 2 例，多発斑状陰影 2 例，その他），HTLV-1 associated myelopathy/tropical spastic paraparesis（HAM/TSP）：2 例（ともに DPB 様陰影），HTLV-1 キャリア：54 例〔DPB 様陰影 16 例（30.0%），UIP 様陰影 3 例（5.6%），気管支拡張 3 例（5.6%），その他〕であった。DPB 様の気道病変がやはり多い結果であったが，UIP あるいは NSIP 様陰影など，DPB 様以外の陰影も少なくはなかった（表，図 2〜7）。

5 考　察

HTLV-1 関連肺病変（気道病変含む）に関しては，ATL 発症者，HTLV-1 キャリアとともに，詳細不明でいまだ混沌とした状態である。HTLV-1 キャリアにおいては，当科の検討でも，これまで報告されてきた DPB 様の気道病変がやはり多い結果であったが，UIP あるいは NSIP 様陰影など，DPB 様以外の陰影も少なくはない。DPB 様所見を合併した HTLV-1 キャリアからの ATL 発症率が高いとの報告があり（当科症例あり。図 3），HTLV-1 キャリアをフォローする際には常に ATL 発症に関しても留意することが大切である

表　HTLV-1 抗体 陽性 74 例の BAL 時の肺野陰影の内訳

ATL 発症：18 例
カリニ肺炎（5 例），DPB 様陰影（2 例），多発斑状陰影（2 例），下葉粒状陰影（1 例），びまん性すりガラス陰影（1 例），肺門陰影増強（1 例），細菌性肺炎（1 例），右中葉腫瘤陰影（1 例），詳細不明（4 例）

HAM/TSP：2 例
ともに DPB 様陰影

HTLV-1 キャリア：54 例
小葉中心性粒状陰影，細気管支炎陰影（＝DPB 様）（16 例，30.0%），UIP 様陰影（3 例，5.6%）
気管支拡張症様陰影（3 例，5.6%），NSIP 様陰影（2 例，3.7%），AIP 様陰影（1 例，1.9%），LIP 様陰影（1 例，1.9%）
その他：
過敏性肺臓炎合併（1 例），慢性好酸球肺炎合併（1 例），器質化肺炎様陰影（1 例），他基礎疾患あり（8 例）
陰影分類難（6 例），陰影詳細不明（3 例），明らかな陰影なし（8 例）
（BAL の施行理由が慢性咳嗽や不明熱，胸水の原因精査など）

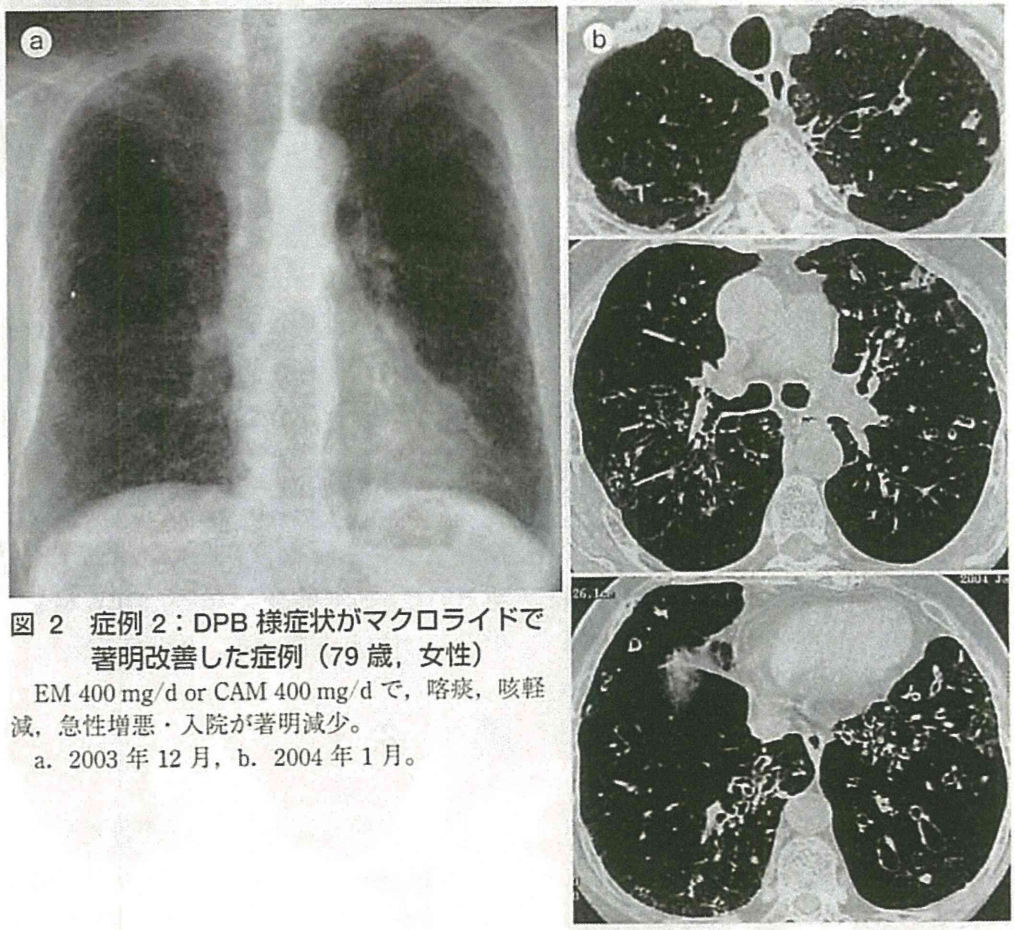

図 2　症例 2：DPB 様症状がマクロライドで著明改善した症例（79 歳，女性）
EM 400 mg/d or CAM 400 mg/d で，喀痰，咳軽減，急性増悪・入院が著明減少。
a．2003 年 12 月，b．2004 年 1 月。

と思われる。HTLV-1 関連気道病変および肺病変の発症病態解析に関しては，今後とも症例の蓄積が必須であり，サイトカインネットワーク，免疫系，および遺伝系などのさらなる詳細な検討が必要と思われる。そして症例の治療に結びついていくことが望まれる。

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