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特集(1)：HTLV-1感染の検査と臨床

2. HTLV-1感染の疫学

高城一郎*1　岡山昭彦*2

〔Key Words〕Human T-lymphotropic virus type-1：HTLV-1(ヒトTリンパ向性ウイルス1型)，adult T-cell leukemia：ATL(成人T細胞白血病)，natural history(自然史)

はじめに

ヒトTリンパ向性ウイルス1型(human T-lymphotropic virus type-1：HTLV-1)は，成人T細胞白血病・リンパ腫(adult T-cell leukemia/lymphoma：ATL)，さらにHTLV-1関連脊髄症(HTLV-1 associated myelopathy：HAM)や眼疾患であるHTLV-1関連ブドウ膜炎(HTLV-1 associated uveitis：HU)などの疾患を引き起こす[1)~3)]。また一部の膠原病や小児の皮膚疾患などとの関連も報告されている[4)5)]。ATLは1977年に高月らにより，新しい疾患概念として最初に報告された[1)]。1981年にはこのウイルスがATLの原因ウイルスであることが日沼らにより明らかにされた[6)]。

HTLV-1感染者(キャリア)の大部分はHTLV-1関連疾患を発症せずに無症状であるが，その一部(約5%)が感染後数十年の長い潜伏期を経てATLを発症する。HAMやHUの頻度はATLよりも少ないと報告されている。本邦では現在でも約108万人のキャリアが存在し，その分布は人口の移動により従来の西南日本のみならず大都市圏にも拡大し，1年間に1000人以上がATLを発症すると推定されている。

HTLV-1キャリアにおいてウイルス粒子そのものは血液や体液中より検出することはできず，主にCD4陽性リンパ球中にプロウイルスの形で存在している。このためHTLV-1感染でいうウイルス量とは多くの場合感染細胞数を意味している。HTLV-1感染は感染細胞(プロウイルス保有細胞)が他者の体内に入り細胞－細胞接触により成立する。新規感染成立後は体内でも細胞間感染により感染が拡大し，HTLV-1に対する免疫の発動後は感染細胞のクローン増殖が感染の維持に大きな役割を果たすと考えられている。感染は母児間ないし配偶者間感染で成立するが，医原的には輸血や臓器移植で起こりうる。感染が成立すると終生持続感染が維持され，自然にウイルスが消失することはないと考えられている。本項ではHTLV-1感染の疫学について述べる。

I. 世界におけるHTLV-1キャリアの地理的分布

世界におけるHTLV-1感染者は約1000～2000万人と推測されるが正確な数は明らかでない。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自体の感染力が非常に

*1,2 Ichiro TAKAJO, MD & Akihiko OKAYAMA, MD
宮崎大学医学部内科学講座 免疫感染病態学分野(〒889-1692 宮崎県宮崎市清武町木原5200)

弱いため浸淫地域は限られている。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り(図1)，そのほかではアジアや中東の一部に抗体陽性者の報告がある[7]。HTLV-1は分子生物学的手法によって3つの大きな系統(1a: コスモポリタン型，1b: 中央アフリカ型，1c: メラネシア型)に分けられる。コスモポリタン型はさらにA(トランスコンチネンタル)，B(日本)，C(西アフリカ)，D(北アフリカ)，E(ペルー)のサブタイプが報告されている[8]。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。HTLV-1のサブグループの違いによる関連疾患の違いは報告されていない。

II. 本邦における疫学

本邦におけるHTLV-1に関する疫学調査は，1980年代に日沼，田島らによって行われ，推定キャリア数120万人，ATL患者数700人/年間と報告された[9]。それ以降本邦におけるHTLV-1キャリア数の変化の詳細については不明であったが，2008～2010年度に厚生労働科学研究班「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」(山口班)が組織され，全国的なHTLV-1キャリアおよび関連疾患の実態調査が行われた。その中で，初回献血者の抗体陽性者の調査から，全国のキャリア数は約108万人と推定され，依然として全国民の1%に相当する多数のキャリアが存在していることが判明し，またキャリアの高齢化が明らかとなっ

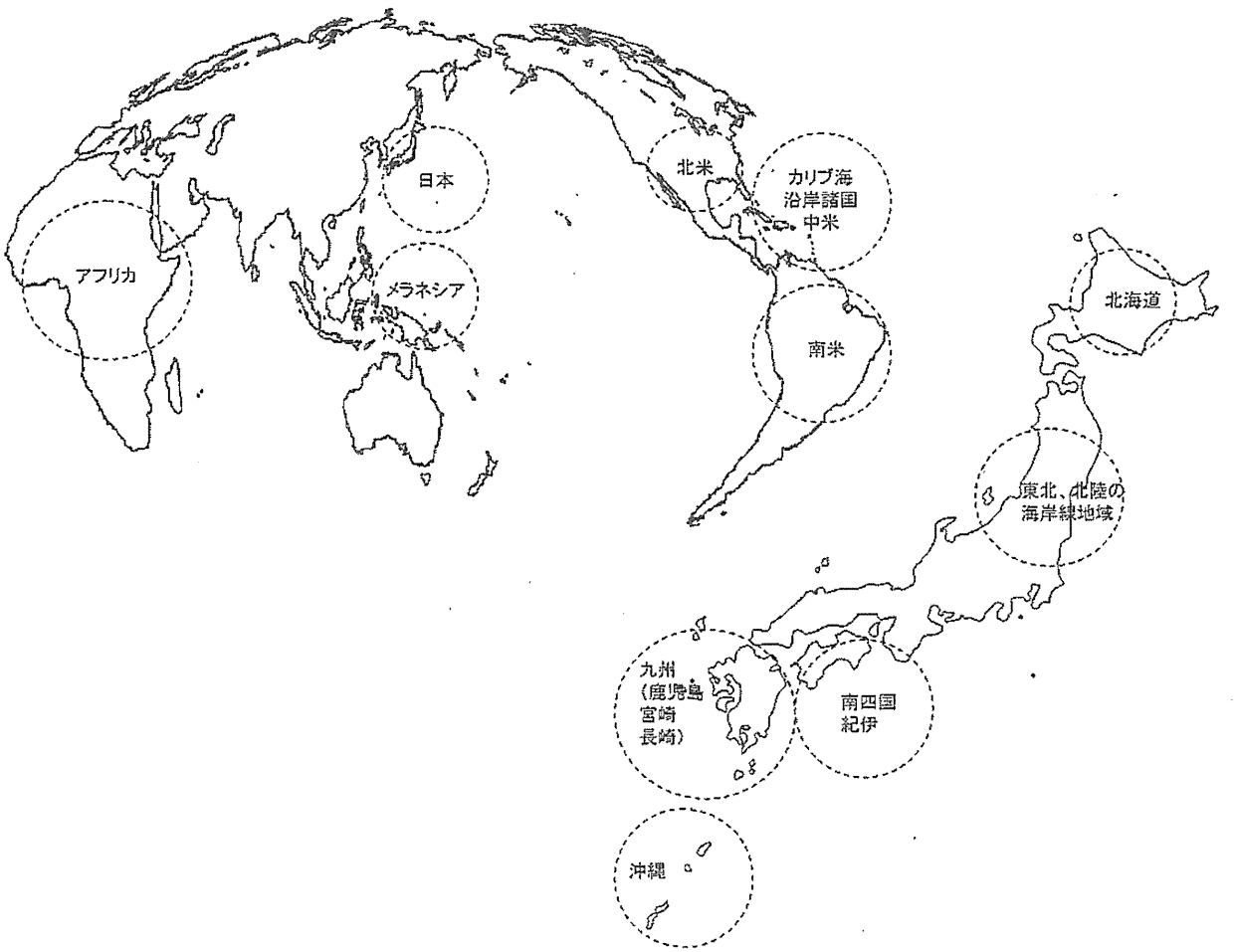

図1　世界におけるHTLV-1の分布と日本国内におけるHTLV-1高浸淫地域

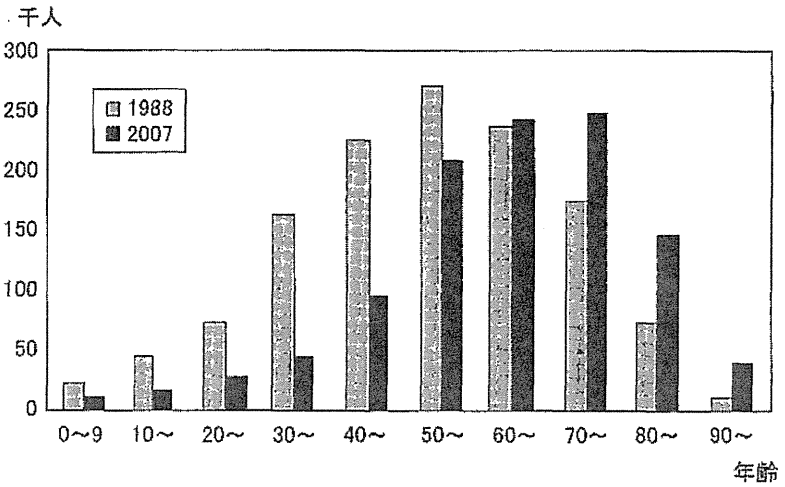

図2　推定されるキャリアの年齢別分布の推移(文献[10]より改変)

た(図2)[10]。ATL の実態調査から ATL 患者数はむしろ増加傾向(1000 人/年間超)にあり，人口の高齢化に伴い，今後も持続的に ATL は発症すると考えられた。1980 年代の疫学調査からは国内においても HTLV-1 キャリアの分布には地域差があり，感染率の高い地域は九州，四国，沖縄などの西南日本が主であるとされた。このほかに東北や北陸地方の一部，特に海岸線地域，また北海道に感染者の割合の比較的高い地域が報告された(図1)[9]。今回の調査で九州・沖縄地方のキャリアの割合が減少している一方，関東地方と近畿地方の大都市圏では増加が認められ，感染が全国に拡散する傾向があることが示された(図3)[10]。

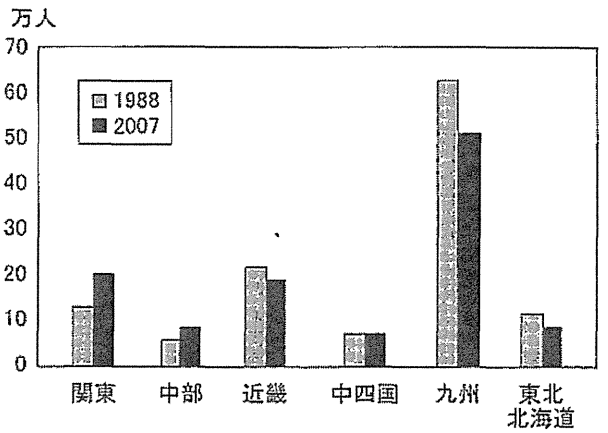

図3　推定される地域別キャリア数の推移
(文献[10]より改変)

HTLV-1 高浸淫地方において，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[11]。これは HTLV-1 の自然感染がおもに家族内に限られることによると思われる。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てきた[12]。年齢が高くなるほど感染率が高くなる理由としては 2 つ考えられた。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の高さは，その人々が出生した年における感染率(おそらく母児感染の頻度)が高かったことを反映しているというものである[13]。もうひとつの理由は配偶者間感染のため一定の年齢以降感染率が高くなるというものである。実際に HTLV-1 感染者の配偶者を長期にフォローすると一定の割合で抗体の陽転が生じる[14]。

III. HTLV-1 の感染経路

HTLV-1 の感染経路としては，母児間感染，配偶者間感染，輸血による感染が知られている[15]。本邦では輸血によるものは献血者の抗体スクリーニングにより 1984 年以降阻止されており，母児間感染が主要な感染経路と考えられている。

母児間感染は主に母乳を介して成立する。キャリアの母親が母乳を与えた場合の児への感染

率は16～30%といわれており，母乳中の感染リンパ球が児への感染に関与していると考えられている[16)17)]。大部分のキャリアはATLを発症しないが，ATLが発症した場合の予後が不良であることを考慮し，将来のATL発症予防のためHTLV-1キャリア妊婦に対しては母乳で哺育しないことが推奨されてきた。長崎県において1987年よりHTLV-1陽性の母親への介入が開始され，母乳哺育を遮断することにより母児間感染の大部分を回避することが可能となった[18)]。その他の対策として人工乳哺育，3ヵ月以内の短期母乳哺育，凍結哺育などの方法があり，児へのHTLV-1感染リスクが減少することが報告されてきた。ただし母乳による哺育を行わない場合でも，児への感染は数%あり，母乳以外の感染経路(子宮内感染あるいは産道感染)があると推定されている。

このようなHTLV-1キャリア妊婦に対する対策は従来，九州・沖縄などHTLV-1キャリアの多い地域を中心に推進されてきたが，山口班の研究によりHTLV-1キャリアが全国に拡散していることが報告された。また平成21年度厚労科研費補助金厚労科学特別研究事業「HTLV-1の母子感染予防に関する研究」(齋藤班)からも全国の妊婦に対してHTLV-1抗体スクリーニング検査の必要性が提起された。このような状況をうけて，2010年秋より母子感染予防の組織的な取り組みが全国的なレベルで行われることとなった。しかし，齋藤班により，特にHTLV-1キャリアが少ない地域でHTLV-1スクリーニング検査(粒子凝集法もしくは化学発光法)を行うと，陽性者の中に偽陽性者が少なからず存在し，確認検査(Western blot法)が必要であること，また確認検査を行っても判定保留(2～20%)となる場合もあることが報告された。このため検査の性質について検査前にキャリア妊婦に説明とカウンセリングを行っておくことの必要性が示されている。また確認検査については，血清学的検査以外の方法の導入をふくめて，検査法のさらなる改善が試みられつつある。

HTLV-1のもうひとつの感染経路は性交渉による配偶者間感染である。配偶者間感染はプロウイルスの遺伝子配列の比較によっても証明されており，男女どちらからでも起こりうるが，夫がキャリアである場合は妻がキャリアである場合に比して4～5倍感染が成立しやすい[19)]。このことは中年以降において女性の抗体陽性率が男性よりも高いことも説明する。配偶者間感染は必ずしも結婚の直後に成立するわけではなく，50歳代，60歳代になって感染することもまれではない。感染してからATLを発症するまでには通常数十年の長い年月がかかるため，配偶者間で感染してもATLが発症することはないと考えられている。しかし，HAMやHUは低率ではあるが発症することがありうる。性交渉による感染は理論的にはコンドーム使用により防ぐことができると考えられるが，挙児希望の場合には確実に感染を予防できる方法はなく，また感染を防ぐワクチンも開発されていない。そのため，将来的には配偶者間感染に関してもなんらかの予防法が検討される必要があると思われる。

配偶者間感染の初期像の解析から，ヒト免疫不全ウイルス同様HTLV-1でも感染後抗体陰性の時期があり，その時期にウイルス量がいったん増加すること，その後の抗体産生後にウイルス量が低下し，一定のレベル(セットポイント)に安定すること，またセットポイントはキャリアごとに異なり，長期間安定の状態にあると考えられている[14)]。さらにジャマイカの母児感染の研究から母児間感染についてもセットポイントは各小児において異なること，10歳前後ですでにHTLV-1感染細胞のクローン増殖をきたす例があり，皮膚病変などの症状を呈することがあることも判明している[20)]。また，キャリアの長期フォロー研究から，感染ウイルス量が多いことはATL発症をきたす危険因子であることも明らかとなっている[21)22)]。これらの知見よりHTLV-1感染の防止の必要性は明らかであるが，感染成立後においてもセットポイントのウイルス量を減少させることが可能であればHTLV-1関連疾患の予防に有効と考えられる。しかし，

現在のところヒト HTLV-1 新規感染に対して母乳遮断以外にワクチンなどの有効な感染予防策は知られておらず，また感染ウイルス量を抑える方法も明らかでない。今後の検討課題であると思われる。

IV. ま と め

本邦において HTLV-1 キャリア数は自然減あるいは母児感染対策により減少してきている。しかし，ATL を含めた HTLV-1 関連疾患を発症するキャリア数は未だに決して少なくなく，解決されるべき問題は多い。今後，HTLV-1 感染を駆逐するような抗ウイルス療法，あるいはウイルス量を低下させる方法論の開発により，関連疾患の発症予防が可能となることが望まれる。

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Interleukin-1 alpha produced by human T-cell leukaemia virus type I-infected T cells induces intercellular adhesion molecule-1 expression on lung epithelial cells

Yuko Nakayama,[1,2] Chie Ishikawa,[1,3] Kazumi Tamaki,[1] Masachika Senba,[4] Jiro Fujita[2] and Naoki Mori[1]

Correspondence
Naoki Mori
naokimori50@gmail.com

[1]Department of Microbiology and Oncology, Graduate School of Medicine, University of the Ryukyus, Nishihara, Okinawa, Japan

[2]Department of Infectious, Respiratory and Digestive Medicine, Graduate School of Medicine, University of the Ryukyus, Nishihara, Okinawa, Japan

[3]Transdisciplinary Research Organization for Subtropics and Island Studies, University of the Ryukyus, Nishihara, Okinawa, Japan

[4]Department of Pathology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan

The pathogenic mechanism of human T-cell leukaemia virus type I (HTLV-I)-related pulmonary disease, which involves overexpression of intercellular adhesion molecule-1 (ICAM-1) in lung epithelial cells, was investigated. The supernatant of HTLV-I-infected Tax$^+$ MT-2 and C5/MJ cells induced ICAM-1 expression on A549 cells, a human tumour cell line with the properties of alveolar epithelial cells. Neutralization of ICAM-1 partially inhibited HTLV-I-infected T-cell adhesion to A549 cells. Analysis of the ICAM-1 promoter showed that the nuclear factor-kappa B-binding site was important for supernatant-induced ICAM-1 expression. Induction of interleukin (IL)-1 alpha (IL-1α) expression in MT-2 and C5/MJ cells was observed compared with uninfected controls and HTLV-I-infected Tax-negative cell lines. The significance of IL-1α as a soluble messenger was supported by blocking the biological activities of MT-2 supernatant with an IL-1α-neutralizing mAb. Moreover, Tax and IL-1α expression was demonstrated in the bronchoalveolar lavage cells of patients with HTLV-I-related pulmonary disease. Immunohistochemistry confirmed ICAM-1 and IL-1α expression in lung epithelial cells and lymphocytes of patients with HTLV-I-related pulmonary diseases, and in a transgenic mouse model of Tax expression. These results suggest that IL-1α produced by HTLV-I-infected Tax$^+$ T cells is crucial for ICAM-1 expression in lung epithelial cells and subsequent adhesion of lymphocytes in HTLV-I-related pulmonary diseases.

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INTRODUCTION

Human T-cell leukaemia virus type I (HTLV-I) is a retrovirus associated with adult T-cell leukaemia (Hinuma *et al.*, 1981) and HTLV-I-associated myelopathy/tropical spastic paraparesis (Gessain *et al.*, 1985; Osame *et al.*, 1986). HTLV-I is also implicated in inflammatory disorders, such as uveitis, arthropathy and Sjögren's syndrome (Watanabe, 1997). Transgenic mice expressing the Tax protein, encoded by the *pX* gene, develop arthropathy (Iwakura *et al.*, 1991) and exocrinopathies affecting the lacrimal and salivary glands (Green *et al.*, 1989). HTLV-I is also associated with pulmonary involvement. Patients with HTLV-I-associated myelopathy/tropical spastic paraparesis and uveitis or asymptomatic carriers exhibit pulmonary complications characterized by T-lymphocyte alveolitis or lymphocytic interstitial pneumonia (Sugimoto *et al.*, 1987, 1993). Furthermore, in Tax-expressing transgenic mice, inflammatory cells consisting of lymphocytes accumulate in peribronchiolar and perivascular areas, as well as in alveolar septa (Miyazato *et al.*, 2000).

It has been estimated that there are 28 000 type I pneumocytes and 1400 type II pneumocytes per alveolus in a human (Crandall & Kim, 1991). Because of its strategic

Abbreviations: AP-1, activator protein-1; BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; BCECF, 2′,7′-bis-(2-carboxyethyl-5)- and 2′,7′-bis-(2-carboxyethyl-6)-carboxyfluorescein, acetoxymethyl ester; EMSA, electrophoretic mobility shift assay; FBS, fetal bovine serum; HBZ, HTLV-I basic leucine zipper domain protein; HTLV-I, human T-cell leukaemia virus type I; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; LFA-1, lymphocyte function-associated antigen-1; MMC, mitomycin C; NF-κB, nuclear factor-kappa B; pAb, polyclonal antibody; TNF-α, tumour necrosis factor alpha.

location, the alveolar epithelium encounters infectious agents and inflammatory molecules. It is thought to play an important role in the immune response by expressing biologically active mediators and adhesion molecules (Diamond *et al.*, 2000).

The pathogenesis of HTLV-I-associated diseases is poorly understood. Viral and host factors, such as proviral load and immune response, are believed to play an important role in the pathogenesis and progression of diseases. $CD4^+$ T cells are the main target of HTLV-I and carry the proviral load (Bangham, 2003; Kinet *et al.*, 2007). In the broncho-alveolar lavage (BAL) fluid (BALF) of carriers, the copy number of proviral DNA correlates with the number of lymphocytes (Mori *et al.*, 2005). The interaction between infected T cells with a different kind of host cell and immune reactions mediated by these infected cells may play a role in the pathogenic process of HTLV-I-associated complications.

Intercellular adhesion molecule-1 (ICAM-1) interacts with its receptor, lymphocyte function-associated antigen-1 (LFA-1), on leukocytes, and plays an important role in the recruitment and migration of immune effector cells to the site of inflammation (Jutila, 1992; Marlin & Springer, 1987). Infected lymphocytes produce no cell-free virions *in vivo*, and a specialized cell–cell contact, termed the virological synapse, is required for transmission of HTLV-I between cells (Igakura *et al.*, 2003). ICAM-1 and LFA-1 play a key role in the formation of the virological synapse (Barnard *et al.*, 2005).

T cells and their mediators are major players in the immune responses to viral infection. During infection, the expression levels of cytokines and chemokines are significantly higher in the BALF of patients with HTLV-I-related pulmonary diseases than in that of healthy subjects (Yamazato *et al.*, 2003). However, little is known regarding how such humoral factors contribute to the pathological changes in HTLV-I-related pulmonary diseases. Here, we evaluated the biological effects of HTLV-I-infected T-cell supernatants on lung epithelial ICAM-1 expression.

METHODS

Reagents. Recombinant human interleukin (IL)-1 alpha (IL-1α), tumour necrosis factor alpha (TNF-α) and IL-17 were purchased from PeproTech. Blocking anti-human IL-1α mAb (clone 4414) and anti-human ICAM-1 polyclonal antibody (pAb) were purchased from R&D Systems.

Cells. The HTLV-I-infected T-cell lines MT-2 (Miyoshi *et al.*, 1981), MT-4 (Yamamoto *et al.*, 1982), C5/MJ (Popovic *et al.*, 1983), HUT-102 (Poiesz *et al.*, 1980), MT-1 (Miyoshi *et al.*, 1980), TL-Oml (Sugamura *et al.*, 1984) and ED-40515(−) (Maeda *et al.*, 1985), and uninfected T-cell lines Jurkat, MOLT-4 and CCRF-CEM were cultured in RPMI 1640 containing 10 % fetal bovine serum (FBS). MT-2, MT-4 and C5/MJ are HTLV-I-transformed T-cell lines established by an *in vitro* co-culture protocol and constitutively express viral genes, including Tax. MT-1, TL-Oml and ED-40515(−) are T-cell lines of leukaemic cell origin that were established from patients with adult T-cell leukaemia but do not express viral genes. HUT-102 was established from a patient with adult T-cell leukaemia, but its clonal origin is unclear.

Preparation of culture supernatants. Human T-cell lines were incubated at a cell density of 1×10^6 cells ml^{-1} for 72 h. The supernatants were stored at −80 °C until use. Dilution of the supernatants from cultured HTLV-I-infected T-cell lines was carried out using RPMI 1640 containing 10 % FBS.

Subjects. The subjects consisted of 11 consecutive HTLV-I infected patients with lung lesions in computed-tomography scan findings and various pulmonary symptoms (chronic cough and/or dyspnoea on effort) who visited our hospital and received BAL analysis. All patients were designated carriers, and included four patients with diffuse panbronchiolitis and three with autoimmune diseases. Two control subjects were also included who were seronegative for HTLV-I. This study was approved by the Institutional Review Board at the University of the Ryukyus.

BAL and cell preparation. BAL was performed in the 11 patients using a standard technique (Yamazato *et al.*, 2003). The BALF obtained was passed through two sheets of gauze. After washing three times with PBS supplemented with 1 % heat-inactivated fetal calf serum, the total number of cells was counted. An aliquot was centrifuged and the supernatants were stored at −80 °C until assayed. The remaining cells were pelleted by centrifugation, mixed with Isogen (Wako Pure Chemical Industries) and stored at −80 °C until use. All subjects provided informed consent before BAL.

Adhesion assay. Human A549, a tumour cell line from a human lung carcinoma with the properties of type II alveolar epithelial cells (Lieber *et al.*, 1976), was plated at a confluent density. C5/MJ cells were suspended in serum-free RPMI 1640 containing 5 μM 2′,7′-bis-(2-carboxyethyl-5)- and 2′,7′-bis-(2-carboxyethyl-6)-carboxyfluorescein, acetoxymethyl ester (BCECF; Dojin Chemicals). After incubation for 45 min at 37 °C, these cells were washed with PBS and resuspended in culture medium. Before adhesion experiments, A549 cells were pre-incubated for 1 h in the presence of either anti-ICAM-1 pAb or control IgG. Subsequently, BCECF-labelled C5/MJ cells (10^5 cells per well) were deposited on MT-2 supernatant-stimulated A549 cell monolayers for 30 min at 37 °C. Each experiment was run in triplicate. Non-adherent cells were removed by inverting the plates and washing with PBS. Adherent cells were solubilized with 1 % NP-40 in PBS, and the fluorescence intensity of each well was measured using an ARVO MX spectrophotometer (PerkinElmer).

HTLV-I infection by co-cultivation. A549 cells were maintained in RPMI 1640 containing 10 % FBS. MT-2 cells (Miyoshi *et al.*, 1981) were used as the HTLV-I-infected T-cell line and produced viral particles. MT-2 cells were treated with 100 μg mitomycin C (MMC) ml^{-1} (Sigma-Aldrich) for 1 h at 37 °C. After washing three times with PBS, they were cultured with an equal number of A549 cells in RPMI 1640 containing 10 % FBS. A549 cells were harvested at 24, 48 and 72 h.

RT-PCR. Total RNA was extracted with TRIzol (Invitrogen) or Isogen (Wako Pure Chemical Industries) according to the protocol provided by the manufacturer. First-strand cDNA was synthesized from 1 μg total cellular RNA using an RNA PCR kit (Takara Bio) with random primers. Thereafter, cDNA was amplified. The sequences of the primers have been described elsewhere (Ansai *et al.*, 2002; Brenner *et al.*, 1989; Hieshima *et al.*, 2008; Liu *et al.*, 2006; Nakayama *et al.*, 2008). The PCR products were fractionated on 2 % agarose gels and assessed qualitatively by (0.5 μg ml^{-1}) ethidium bromide staining.

Immunofluorescence staining. Fixed cells were washed with PBS containing 7 % FBS and permeabilized with PBS containing 0.1 %

Triton X-100. The cells were washed with PBS/7% FBS and resuspended in PBS/7% FBS containing mouse mAb against Tax (Lt-4; Tanaka *et al.*, 1990). The cells were washed with PBS/7% FBS and resuspended in PBS/7% FBS containing Alexa Fluor 488-labelled goat anti-mouse IgG (Invitrogen). The nuclei were stained with Hoechst 33342 (Wako Pure Chemical Industries). Finally, the cells were washed with PBS/7% FBS and observed under a DMI6000 microscope (Leica Microsystems).

Immunohistochemical staining. We examined lung biopsy specimens from three patients with HTLV-I-related pulmonary diseases, and lung biopsy specimens from transgenic mice bearing Tax or control littermate mice (Iwakura *et al.*, 1991). All human subjects provided informed consent before samples were obtained. The tissue samples were subjected to immunohistochemical staining using mouse mAbs to ICAM-1 (clone 15.2) and IL-1α (clone B-7) (Santa Cruz Biotechnology). Sections were counterstained with methyl green.

Flow cytometry. To measure the expression of ICAM-1 and LFA-1 on the surface of A549 cells after the addition of culture supernatants or after HTLV-I infection, FITC-labelled mouse mAb against ICAM-1 (CD54, clone 84H10), LFA-1 α-chain (CD11a, clone 25.3) or control mouse IgG1 (Coulter Immunotech) was used. Cells were analysed on an Epics XL flow cytometer (Beckman Coulter).

Measurement of IL-1α. IL-1α levels in culture supernatants and BALF were measured by ELISA (Invitrogen) following the procedure recommended by the manufacturer. A sufficient quantity of BALF was available from HTLV-I carriers to estimate the level of IL-1α after concentrating the supernatant using a Centricon Plus-70 centrifugal filter (Millipore), which is used to concentrate low-molecular-mass components with a cut-off of 5000 Da. As BAL has a diluting effect on the recovery of IL-1α, measurements were standardized against albumin.

Reporter assay. A549 cells were transfected with luciferase reporter constructs for the ICAM-1 promoter (Ledebur & Parks, 1995) using Lipofectamine (Invitrogen). After 24 h, the transfected A549 cells were cultured in the presence or absence of MT-2 supernatant for 24 h before the luciferase assay. For reporter assays, a nuclear factor-kappa B (NF-κB) site-dependent luciferase vector, κB-LUC (Suzuki *et al.*, 1995), and an expression plasmid for Tax (Matsumoto *et al.*, 1997) were also used. Luciferase activities were measured using a dual luciferase assay system (Promega) and normalized against *Renilla* luciferase activity from plasmid phRL-TK (Promega).

Electrophoretic mobility shift assay (EMSA). Nuclear extracts were obtained as described by Antalis & Godbolt (1991) with modifications, and EMSA was performed as described by Mori & Prager (1996). Briefly, 5 μg nuclear extract was incubated with ^{32}P-labelled probes. The DNA–protein complex was separated from the free oligonucleotides on a 4% polyacrylamide gel. For competition experiments, the cold oligonucleotide probe or competitors were used, and supershift analysis was performed using rabbit pAbs against the NF-κB subunits p50, p65, c-Rel, p52 and RelB (Santa Cruz Biotechnology). The probes used were an NF-κB element of the ICAM-1 gene (5′-tcgaTAGCTTGGAAATTCCGGAGC-3′), a typical NF-κB element from the IL-2 receptor α chain (IL-2Rα) gene (5′-gatcCGGCAGGGGAATCTCCCTCTC-3′) and an activator protein-1 (AP-1) element of the IL-8 gene (5′-gatcGTGATGACTCAGGTT-3′). Underlined sequences represent the NF-κB- or AP-1-binding site, and lower-case letters indicate residues added for labelling purposes.

Statistical analysis. Data are expressed as means ± SD. Statistical difference was analysed using Student's *t*-test. *P* values of <0.05 were considered significant.

RESULTS

Effect of supernatants of HTLV-I-infected and uninfected T cells on the induction of ICAM-1 expression

We first examined the effects of the supernatant from HTLV-I-infected cell culture on A549 cells. The culture supernatants of HTLV-I-uninfected T-cell lines (Jurkat, MOLT-4 and CCRF-CEM) had no substantial effect on A549 cells. In contrast, the culture supernatants of MT-2 and C5/MJ cell lines induced the mRNA expression of ICAM-1 (Fig. 1a). ICAM-1 mRNA was detected at 1 h after the initiation of culture and remained elevated for at least 6 h following incubation compared with the control (Fig. 1b). The effects of the MT-2 and C5/MJ supernatants on A549 cells were observed at a concentration as low as 6% (Fig. 1c). Among the HTLV-I-infected T-cell lines, the culture supernatants of MT-4 and HUT-102 cell cultures and the Tax-negative HTLV-I-infected T-cell lines MT-1, TL-Omi and ED-40515(−) had no substantial effect on A549 cells.

Flow cytometry studies indicated that A549 cells started to express a significant amount of ICAM-1 at 2 or 3 h after the initiation of exposure to C5/MJ or MT-2 supernatant. However, LFA-1 expression on A549 cells was not induced by MT-2 supernatant. ICAM-1 expression remained at a plateau level at 24 h (Fig. 2a). The cell-surface expression of ICAM-1 was also significantly increased after the addition of IL-1α and TNF-α for 3 h (Fig. 2a). In contrast, its expression on A549 cells was not enhanced in cultures with Jurkat supernatant. The dose dependency of MT-2 supernatant was observed in A549 cells. Consistent with the results of RT-PCR, the highest level of ICAM-1 expression was observed after exposure to 6% MT-2 supernatant (Fig. 2b).

Involvement of ICAM-1 in HTLV-I-infected T-cell adhesion to A549 cells

We examined the surface expression of LFA-1, the major counterligand of ICAM-1, on human T-cell lines by flow cytometry. Jurkat, an HTLV-I-uninfected T-cell line, and the Tax-negative HTLV-I-infected T-cell lines did not express LFA-1. However, of the four Tax$^+$ HTLV-I-infected T-cell lines, three (MT-4, C5/MJ and HUT-102) expressed LFA-1 (Fig. 3a). We next investigated the potential role of ICAM-1 in HTLV-I-infected T-cell adhesion to A549 cells. An anti-human ICAM-1 pAb that blocks cell adhesion was pre-incubated with A549 cells exposed to MT-2 supernatant. One hour after the addition of ICAM-1 pAb, fluorescently labelled C5/MJ cells were added to the A549 culture, and the adherence of C5/MJ cells was measured after 30 min. As indicated in Fig. 3(b), the adhesion of C5/MJ cells to MT-2 supernatant-exposed A549 cells was reduced by the ICAM-1-blocking pAb, suggesting that ICAM-1 acts, at least in part, on HTLV-I-infected T-cell adhesion to lung epithelial cells.

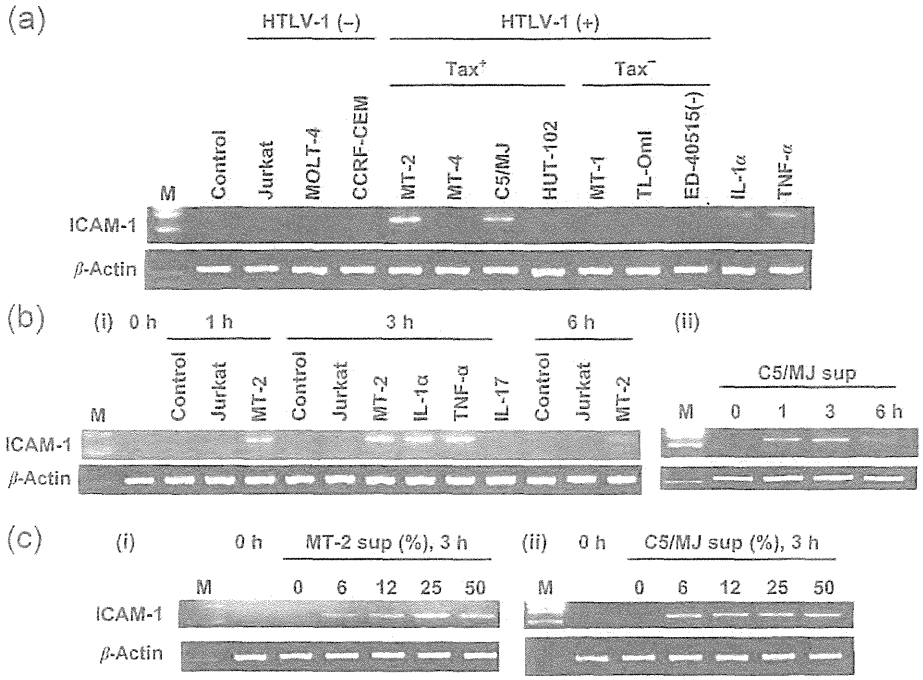

Fig. 1. Effect of the supernatant of HTLV-I-infected and uninfected T cells on mRNA expression of ICAM-1 in A549 cells. (a) MT-2 and C5/MJ supernatants induce ICAM-1 mRNA expression in A549 cells. Total RNA was extracted from A549 cells cultured with 10% supernatant of the indicated cell lines, or with IL-1α (10 ng ml^{-1}) or TNF-α (10 ng ml^{-1}), for 3 h and used for RT-PCR. (b) Time course of expression of ICAM-1 mRNA in A549 cells exposed to MT-2 (i) and C5/MJ (ii) supernatant (sup). Total RNA was extracted from A549 cells cultured with 10% supernatant of the indicated cell lines, or with IL-1α (10 ng ml^{-1}), TNF-α (10 ng ml^{-1}) or IL-17 (10 ng ml^{-1}), for various time intervals and used for RT-PCR. (c) Effect of HTLV-I-infected cell supernatant on A549 cells. Total RNA was extracted from A549 cells exposed to serial 1 : 2 dilutions of MT-2 (i) and C5/MJ (ii) supernatant for 3 h and used for RT-PCR. Human β-actin mRNA was used as a control in all experiments. M, 100 bp ladder (Toyobo Life Science).

Activation of the ICAM-1 promoter by MT-2 supernatant

To confirm that MT-2 supernatant-induced ICAM-1 upregulation is mediated by activation of gene transcription, the ICAM-1 5′-flanking region was analysed with promoter/reporter gene constructs. A549 cells were transiently transfected with a reporter gene construct containing 1353 nt of the ICAM-1 upstream regulatory sequences (pGL1.3). Exposure of MT-2 supernatant caused a 25-fold increase in the activity of this ICAM-1-driven reporter construct in A549 cells, whereas the negative-control vector, pGL2-Basic, showed only low background activity (Fig. 4a). TNF-α- and IL-1β-induced activation of the ICAM-1 promoter has been reported to require the NF-κB site (nt −187 to −178; Hou *et al.*, 1994; Ledebur & Parks, 1995). To determine the functional importance of the NF-κB site, transfections of mutant ICAM-1 promoter/luciferase reporter gene construct with specific mutations in the NF-κB binding site (designated pGL1.3κB^{-}; Ledebur & Parks, 1995) were carried out (Fig. 4a). Mutation of the NF-κB site in the ICAM-1 promoter significantly reduced MT-2 supernatant-mediated luciferase activity in A549 cells. These experiments indicated that the response to MT-2 supernatant stimulation required an intact binding site for NF-κB.

We investigated the induction and binding of nuclear factors to the NF-κB-binding sequence in the ICAM-1 promoter region by MT-2 and C5/MJ supernatants. As shown in Fig. 4(b), a complex formed with the ICAM-1 NF-κB-binding site oligonucleotide probe was induced in A549 cells exposed to MT-2 and C5/MJ culture supernatants. This binding activity was reduced by the addition of cold probe or the IL-2 receptor α chain gene NF-κB-binding site, but not by an oligonucleotide containing the AP-1-binding site (Fig. 4c, lanes 2–4). We also characterized the supernatant-induced complex identified by the ICAM-1 NF-κB-binding site probe. This complex was supershifted by the addition of anti-p50 or anti-p65 antibodies (Fig. 4c, lanes 5 and 6), suggesting that the supernatant-induced ICAM-1 NF-κB-binding activity is composed of p50 and p65. Therefore, the supernatants induced ICAM-1 gene expression, at least in part, through the induced binding of p50 and p65 to the NF-κB-binding site in the ICAM-1 promoter region.

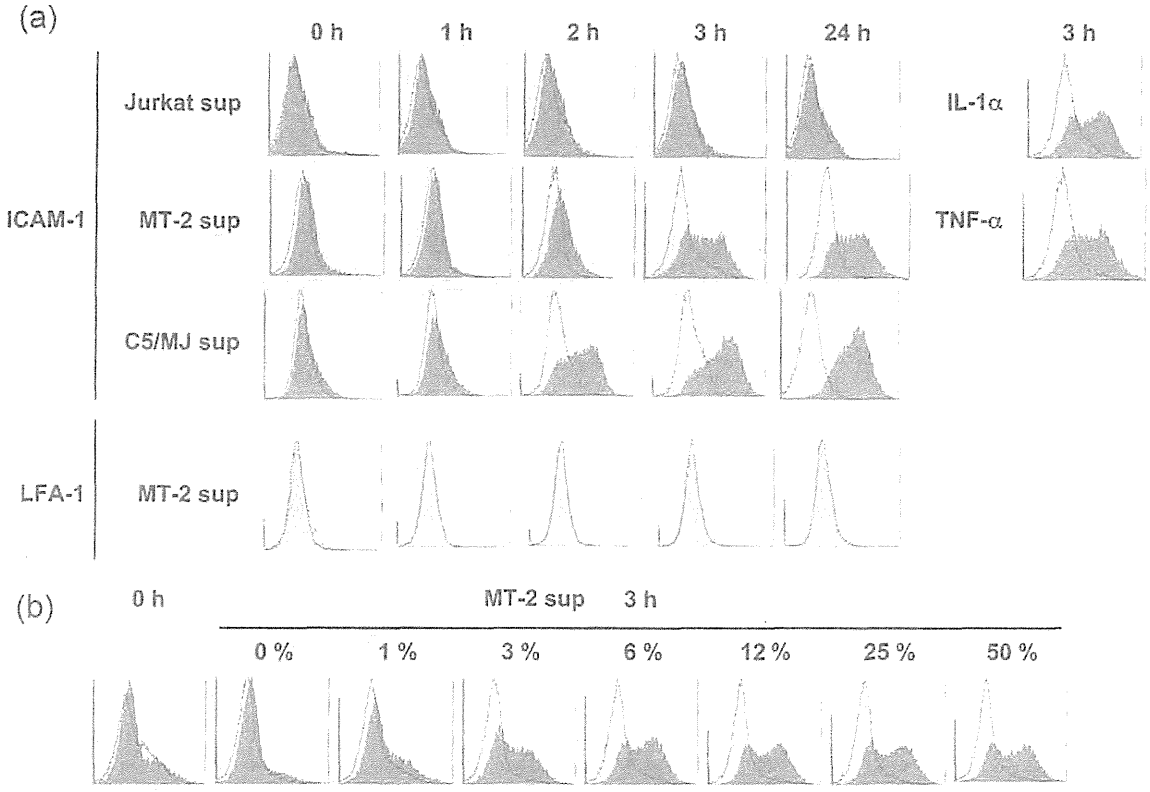

Fig. 2. Supernatants of HTLV-I-infected T cells increase cell-surface expression of ICAM-1 on A549 cells. (a) Time course of cell-surface expression of ICAM-1 on A549 cells exposed to supernatants. A549 cells were cultured for the indicated time intervals in the presence of 10% supernatant of the indicated cell lines, IL-1α (10 ng ml^{-1}) or TNF-α (10 ng ml^{-1}). After cell harvest, ICAM-1 and LFA-1 expression on the cells was determined by flow cytometry. (b) MT-2 supernatant increases cell-surface expression of ICAM-1 on A549 cells in a dose-dependent fashion. A549 cells were exposed to varying concentrations of supernatant of MT-2 cells, and ICAM-1 levels were measured by flow cytometry on cells harvested after 3 h.

IL-1α mediates most of the supernatant-induced ICAM-1 expression

HTLV-I-infected T-cell lines are known to express various types of cytokine, including IL-1α, IL-1β and TNF-α, all of which are known to be strong inducers of ICAM-1 in various types of cells through NF-κB activation (Hou *et al.*, 1994; Ledebur & Parks, 1995; Springer, 1990). To test whether some of these cytokines might be responsible for the induction of ICAM-1 in A549 cells, we examined the mRNA expression levels of IL-1α, IL-1β and TNF-α by RT-PCR. As shown in Fig. 5(a), the pattern of cytokine expression varied among the HTLV-I-infected T-cell lines tested. Low levels of IL-1β mRNA were expressed in C5/MJ cells. Expression of TNF-α mRNA was increased in Tax^+ HTLV-I-infected T-cell lines (MT-2, MT-4, C5/MJ and HUT-102). In contrast, consistent with the induction of ICAM-1 expression, high expression levels of IL-1α mRNA were detected in MT-2 and C5/MJ cells.

We next measured IL-1α levels in the culture supernatants using ELISA. As shown in Fig. 5(b), IL-1α was secreted in MT-2 and C5/MJ cells. To examine whether the effects of the MT-2 supernatant were caused by IL-1α, we performed a blocking test using an anti-IL-1α mAb. The inducible effect of the MT-2 supernatant on ICAM-1 mRNA expression in A549 cells was almost completely blocked by anti-IL-1α mAb (Fig. 5c). Similar to the mRNA expression, the inducible activity of MT-2 supernatant on the cell-surface expression of ICAM-1 was substantially blocked by anti-IL-1α mAb (Fig. 5d).

HTLV-I infection also induces ICAM-1 expression in A549 cells

To determine whether HTLV-I infection of A549 cells upregulated the expression of ICAM-1, A549 cells were co-cultivated with MMC-treated MT-2 cells. After co-cultivation for 2 days, the A549 cells were washed extensively and harvested for assessment by RT-PCR of expression of HTLV-I viral antigen. As the MT-2 cells had been pre-treated extensively with MMC, which causes cell death, no discernible MT-2 cells were seen. Trypan blue staining confirmed the lack of viable MT-2 cells. This finding, together with repeated washing, ensured that no MMC-treated MT-2 cells were present in the A549 culture at the

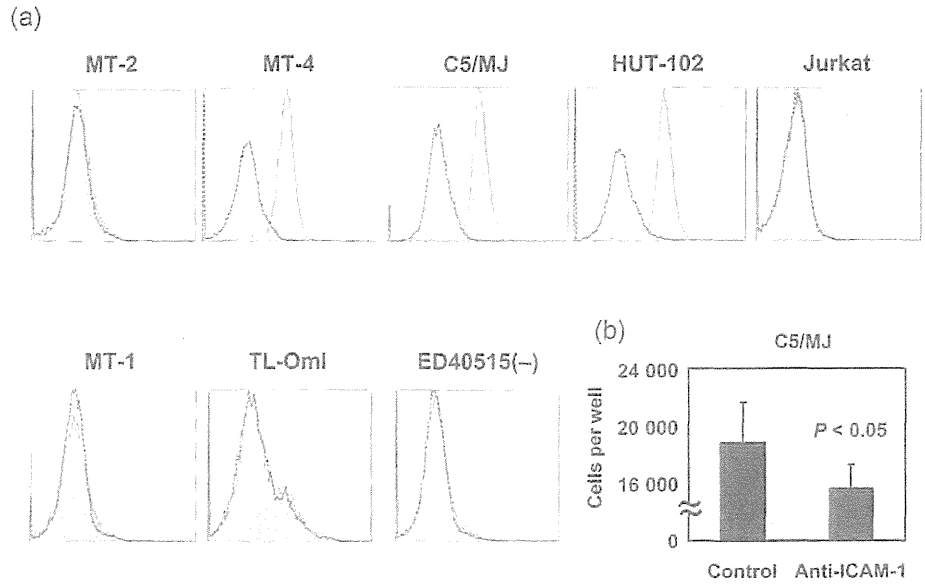

Fig. 3. ICAM-1 is involved in HTLV-I-infected T-cell adhesion to A549 cells. (a) Flow cytometry analysis of surface expression of the LFA-1 α-chain in human T-cell lines. Cells were reacted with FITC-labelled anti-LFA-1 α-chain or an isotype control antibody. (b) An anti-human ICAM-1 pAb was used to block ICAM-1 on the surface of A549 cells. After 1 h, fluorescently labelled C5/MJ cells were added to A549 cells that had been exposed to MT-2 supernatant for 6 h to determine adhesion. After cell lysis, the fluorescence intensity was measured. Data are means ± SD of three experiments.

time of RNA isolation. To examine whether HTLV-I infection had occurred in the infected A549 cells, expression of viral mRNA for Tax and the HTLV-I basic leucine zipper domain protein (HBZ) was assessed by RT-PCR. As shown in Fig. 6(a), A549 cells co-cultured with MT-2 cells showed strong expression of Tax and HBZ mRNA in an MT-2 dose-dependent manner. To further exclude the possibility that viral gene amplification was due to contamination from residual MT-2 cells, we used RT-PCR to amplify MT-2-specific human CD4. As shown in Fig. 6(a), human CD4 product was amplified from MT-2 cells but not from A549 cells co-cultured with MT-2 cells. These results suggested that A549 cells were infected by HTLV-I after co-culture.

To determine whether viral antigens were produced in infected A549 cells, immunofluorescence assays for Tax were performed by indirect immunofluorescence staining. Expression of Tax was detected in the cytoplasm and nuclei of A549 cells at 3 days after HTLV-I infection (Fig. 6b), whereas no Tax expression was observed when a control IgG was used or in control A549 cells that were not co-cultured with MT-2 (data not shown). These observations again demonstrated that HTLV-I infected the A549 cells.

Next, we examined the expression of ICAM-1 mRNA in co-cultured A549 cells by RT-PCR. As shown in Fig. 6(a), ICAM-1 mRNA expression in A549 cells increased substantially 2 days after co-culture with MT-2 cells in an MT-2 dose-dependent manner. However, transcripts of IL-1α were not detected in any of the samples. The surface expression of ICAM-1 on co-cultured A549 cells was also examined by flow cytometry. As shown in Fig. 6(c), ICAM-1 expression on A549 cells increased 1 day after co-culture with MT-2 cells, reached a peak level on day 2 and plateaued on day 3. However, the levels of HTLV-I-upregulated ICAM-1 expression were less than those following exposure to supernatant of HTLV-I-infected T-cell lines. Upregulation of ICAM-1 expression was not observed in A549 cells co-cultured with MMC-treated Jurkat cells or in untreated A549 cells (Fig. 6c).

Tax is the primary viral transactivator protein, modulating the expression of both viral and cellular genes. To examine the effect of Tax on ICAM-1 expression at the transcriptional level, we performed luciferase reporter assays in A549 cells using an ICAM-1 promoter/luciferase reporter plasmid (pGL1.3). As shown in Fig. 6(d), co-transfection of an expression vector for Tax activated the ICAM-1 promoter in A549 cells, indicating that Tax can directly activate the ICAM-1 promoter.

To determine the precise regions of the ICAM-1 promoter necessary for Tax-mediated activation, an NF-κB site mutant (pGL1.3κB$^-$) was co-transfected. Mutation of the NF-κB site abolished the Tax-mediated activation of the ICAM-1 promoter (Fig. 6d). In addition, we confirmed that Tax activated the NF-κB site using the luciferase reporter plasmid regulated by NF-κB elements (κB-LUC; Fig. 6d). These results suggested that Tax transactivates the ICAM-1 gene via the NF-κB-binding site.

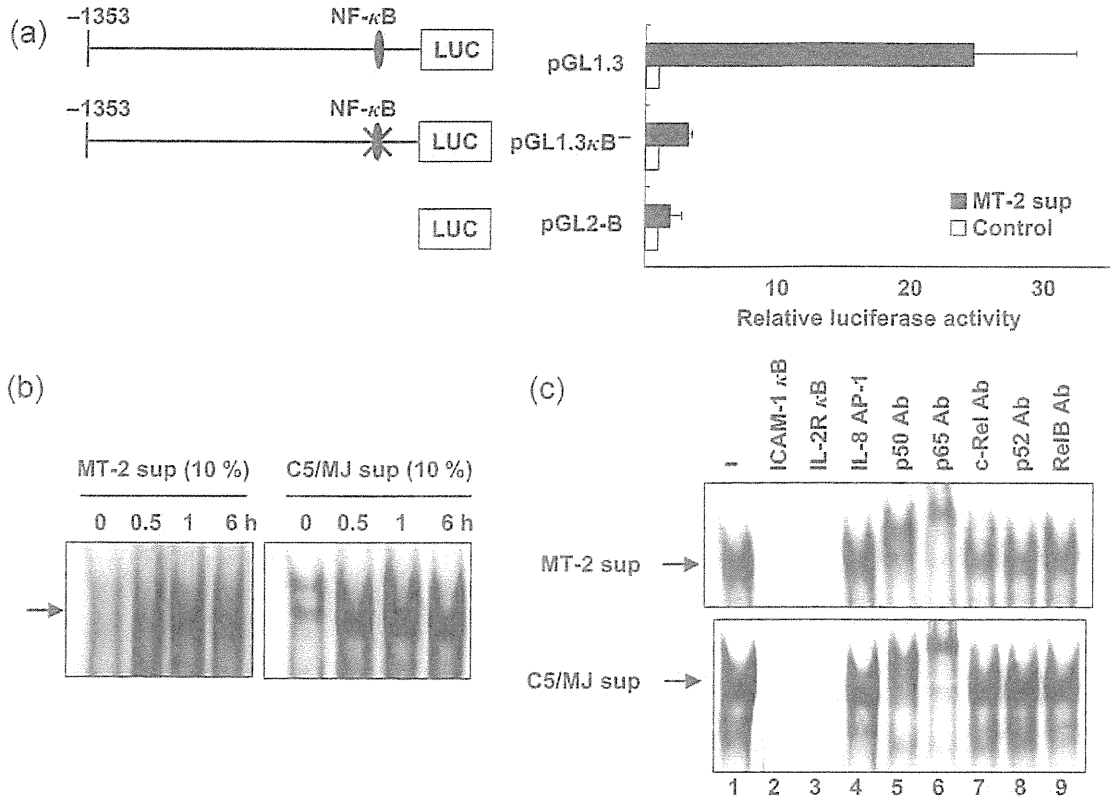

Fig. 4. Identification of a critical supernatant-responsive region of the ICAM-1 promoter. (a) Mutation of the NF-κB site suppresses supernatant-induced ICAM-1 promoter activity. The indicated constructs (left) were transfected into A549 cells and the cells subsequently exposed to MT-2 supernatant for 6 h. The activities (right) of each construct are expressed relative to that of cells transfected with pGL2-Basic (pGL2-B) without further treatment, which was defined as 1. Data are means ± SD of three experiments. (b) Time course of NF-κB activation in A549 cells exposed to MT-2 and C5/MJ culture supernatants was evaluated using EMSA. Nuclear extracts prepared at the indicated time points from A549 cells exposed to the different supernatants were mixed with ICAM-1 NF-κB-binding site labelled probe. The complex is indicated by an arrow. (c) Sequence specificity of NF-κB-binding activity and characterization of NF-κB proteins that bind to the NF-κB-binding site of the ICAM-1 gene. Competition assays were carried out with nuclear extracts from A549 cells exposed to MT-2 supernatant for 0.5 h or C5/MJ supernatant for 1 h. Lane 1 shows the complex formed in the absence of competing oligonucleotide or antibody (indicated by an arrow). Where indicated, a 100-fold excess of a specific competitor oligonucleotide (lanes 2–4) was added to the reaction mixture with the labelled probe. A supershift assay of the NF-κB DNA-binding complex in the same nuclear extracts was also carried out where appropriate antibodies were added to the reaction mixture (lanes 5–9) as indicated.

Detection of IL-1α and ICAM-1 in the lungs of patients with HTLV-I-related pulmonary diseases and in Tax transgenic mice

RT-PCR was used to determine the expression of viral mRNAs of Tax and HBZ in BALF cells from patients with HTLV-I-related pulmonary diseases. In all six patients, Tax and HBZ mRNAs were detected in BALF cells (Fig. 7a). IL-1α mRNA was also detectable in BALF cells. Fig. 7(b) shows the concentrations of IL-1α in BALF samples obtained from HTLV-I carriers and non-infected volunteers. The concentration of IL-1α was higher in four of the HTLV-I carriers than in non-infected controls.

Finally, we immunostained lung tissues obtained from patients with HTLV-I-related pulmonary diseases. The expression of IL-1α and ICAM-1 was noted in the epithelial cells, lymphocytes and macrophages of these patients (Fig. 7c). We also immunostained the lung tissues of transgenic mice to assess the expression of IL-1α and ICAM-1. We examined the distribution of IL-1α and ICAM-1 proteins in the lungs of transgenic mice. Strong immunostaining for IL-1α and ICAM-1 was observed in epithelial cells, lymphocytes and macrophages in the lungs of transgenic mice, but not in the lungs of littermate mice (Fig. 7d).

DISCUSSION

We have been interested in the role of humoral factors released from HTLV-I-infected T cells in the development of the characteristic pathological changes of HTLV-I-associated diseases. In this study, we hypothesized that

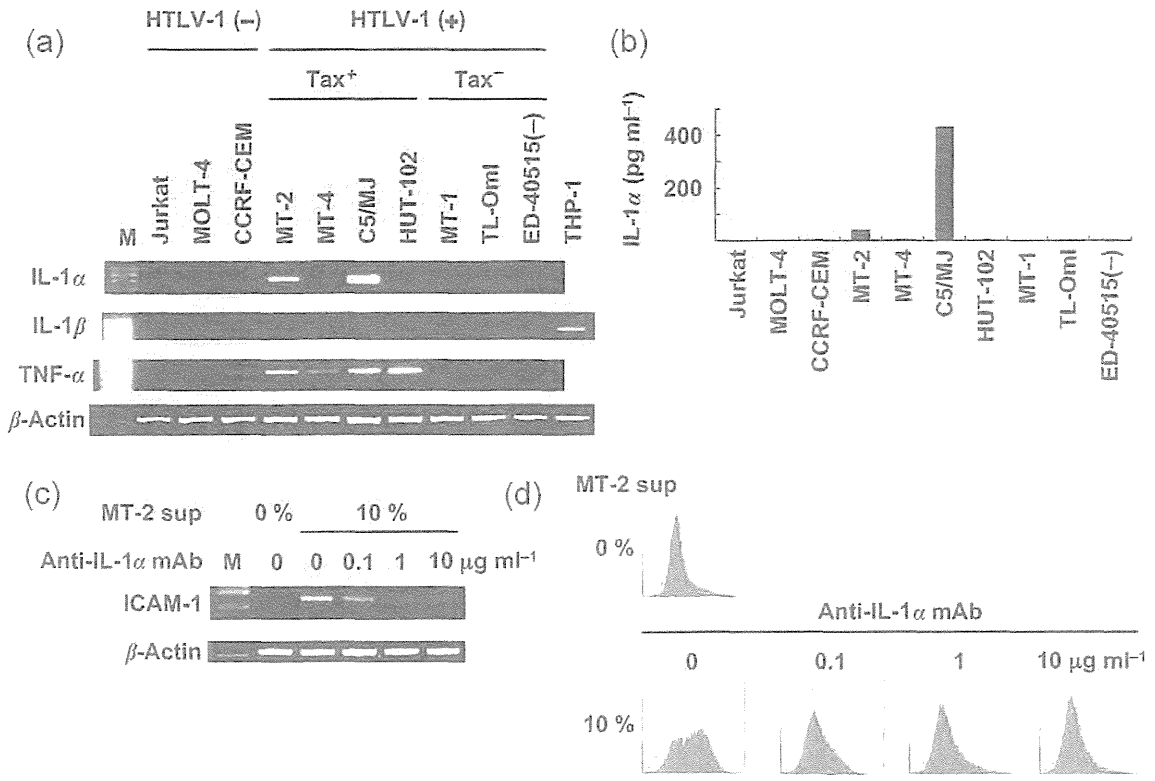

Fig. 5. IL-1α mediates most of the supernatant-induced ICAM-1 expression. (a) Expression of IL-1α, IL-1β and TNF-α mRNAs in various human T-cell lines was examined by RT-PCR. RNA prepared from THP-1 cells was used as a positive control for IL-1β. Human β-actin mRNA was used as a control. M, 100 bp ladder (Toyobo Life Science). (b) Levels of IL-1α secreted by human T-cell lines. Cells were cultured for 72 h and the culture supernatants were collected. The level of IL-1α was determined by ELISA. (c) Effect of neutralizing anti-IL-1α mAb on MT-2 supernatant-induced ICAM-1 mRNA expression in A549 cells. A549 cells were left untreated or were exposed to 10% MT-2 supernatant, cultured with or without the indicated concentrations of neutralizing mAb to IL-1α for 3 h. Human β-actin mRNA was used as a control. (d) The effect of neutralizing anti-IL-1α mAb on MT-2 supernatant-induced ICAM-1 expression in A549 cells was measured by flow cytometry. A549 cells were left untreated or exposed to 10% MT-2 supernatant, cultured with or without the indicated concentrations of neutralizing mAb to IL-1α for 3 h and assessed for cell-surface expression of ICAM-1.

ICAM-1 is strongly expressed on lung epithelial cells of patients with HTLV-I-related pulmonary diseases. In testing this hypothesis, we also examined whether the upregulation of ICAM-1 was mediated by paracrine signalling (i.e. by cytokines secreted from HTLV-I-infected T cells) or directly as a result of HTLV-I infection. The results demonstrated that the culture supernatants of HTLV-I-infected T-cell lines induced the expression of cell-surface ICAM-1 on A549 cells. The culture supernatants activated the ICAM-1 promoter via the NF-κB-binding site. Furthermore, HTLV-I infection of A549 cells also upregulated ICAM-1. However, the levels of ICAM-1 upregulated by HTLV-I infection were less than those following exposure to the supernatants of HTLV-I-infected T-cell lines. HTLV-I Tax alone could also activate the ICAM-1 promoter via the NF-κB-binding site.

It is well known that HTLV-I-infected T cells produce various cytokines, such as IL-1 and tumour necrosis factor (Tschachler *et al.*, 1989; Wano *et al.*, 1987). Our results showed that the main molecule responsible for the biological activity of the culture supernatants was IL-1α for the following reasons: (i) the levels of IL-1α were remarkably high in the MT-2 and C5/MJ supernatants; (ii) adding mAb to IL-1α reduced ICAM-1 expression almost completely; and (iii) the effects of recombinant IL-1α on A549 cells were similar to those of the culture supernatants from HTLV-I-infected T cells. Although MT-2 and C5/MJ cells expressed TNF-α mRNA, HTLV-I-infected T-cell lines did not produce biological TNF-α (Tschachler *et al.*, 1989). These findings suggest that IL-1α was responsible for the biological activities of the supernatants observed in the present study.

With regard to pulmonary lesions, the results demonstrated the presence of high levels of IL-1α in BALF and IL-1α mRNA expression in BALF cells in HTLV-I carriers. We are currently investigating the relationship between IL-1α concentrations and percentages of lymphocytes in the BALF of HTLV-I carriers. In this study, ICAM-1 and IL-1α were detected in lung epithelial cells and lymphocytes,

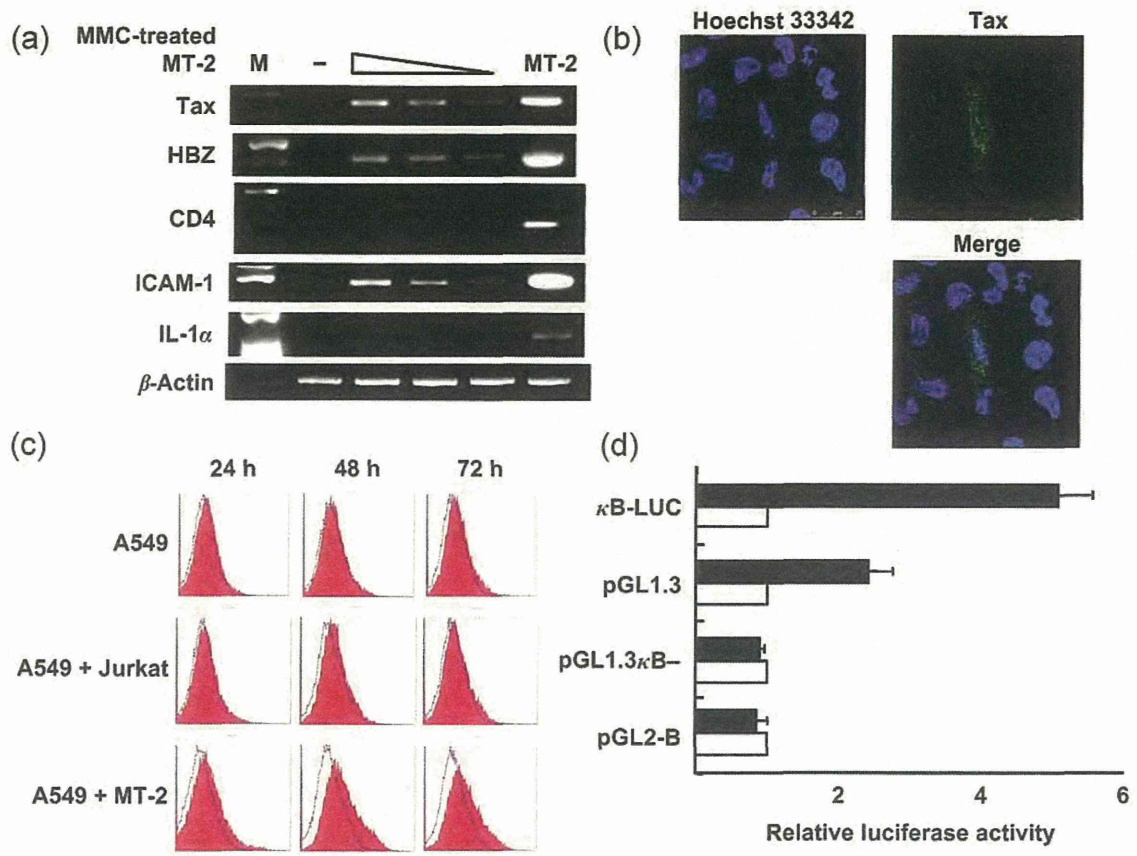

Fig. 6. HTLV-I infection induces ICAM-1 expression in A549 cells. (a) Detection of HTLV-I Tax, HBZ and ICAM-1 mRNAs in A549 cells by RT-PCR. A549 cells were co-cultured with MMC-treated MT-2 cells. After co-cultivation for 2 days, the A549 cells were harvested and expression of the indicated genes was analysed. Human β-actin mRNA was used as a control. M, 100 bp ladder (Toyobo Life Science). (b) Detection of HTLV-I Tax by immunofluorescence. A549 cells were co-cultured with MT-2 cells, fixed and incubated with an anti-Tax antibody, followed by an Alexa Fluor 488-labelled anti-mouse secondary antibody, together with Hoechst 33342 staining of the nuclei. (c) ICAM-1 expression on A549 cells is upregulated after co-culture with MT-2 cells. A549 cells were co-cultured with or without MMC-treated MT-2 or Jurkat cells for 1, 2 and 3 days. Cells were collected and flow cytometry was performed to detect ICAM-1 expression on the cell surface. (d) Tax activates the ICAM-1 promoter via NF-κB. A549 cells were transfected with the indicated constructs together with a Tax expression vector or empty vector. Cells were harvested at 24 h post-transfection and luciferase activity was measured. Activity is expressed relative to that of cells transfected with the reporter construct together with empty vector, which was defined as 1. Data are means ± SD of three experiments. Black bars, Tax; white bars, vector.

respectively, from patients with HTLV-I-related pulmonary diseases. However, ICAM-1 was also detected in lymphocytes and macrophages, and IL-1α was also detected in lung epithelial cells and macrophages. In the lungs of patients with pulmonary disorders associated with HTLV-I, HTLV-I-infected T cells and lung epithelial cells may produce various types of pro-inflammatory cytokines, resulting in upregulation of ICAM-1 and IL-1α in these cells. The concentration of soluble ICAM-1 in the BALF of patients with HTLV-I-associated pulmonary disorders has been reported to be significantly higher than that in non-infected healthy control subjects (Seki *et al.*, 2000). In accordance with our hypothesis, the concentration of soluble ICAM-1 correlated well with the percentage of activated T cells (Seki *et al.*, 2000).

Because ICAM-1 is an attractive target for therapeutic intervention based on its involvement in the inflammatory and viral infectious processes, understanding the precise mechanisms by which its expression is regulated is important. The present study found an HTLV-I-infected cell supernatant-responsive and Tax-responsive region in the ICAM-1 gene containing an NF-κB site. NF-κB regulates the expression of IL-1α (Mori & Prager, 1996). Accordingly, NF-κB is an attractive target for treatment of HTLV-I-associated pulmonary disorders. However, the addition of ICAM-1-blocking pAb inhibited, at least in part, HTLV-I-infected T-cell adhesion to lung epithelial cells. The partial blockade of cell-to-cell adhesion by ICAM-1-blocking pAb suggests the possible effects of another adhesion molecule, in addition to ICAM-1, that

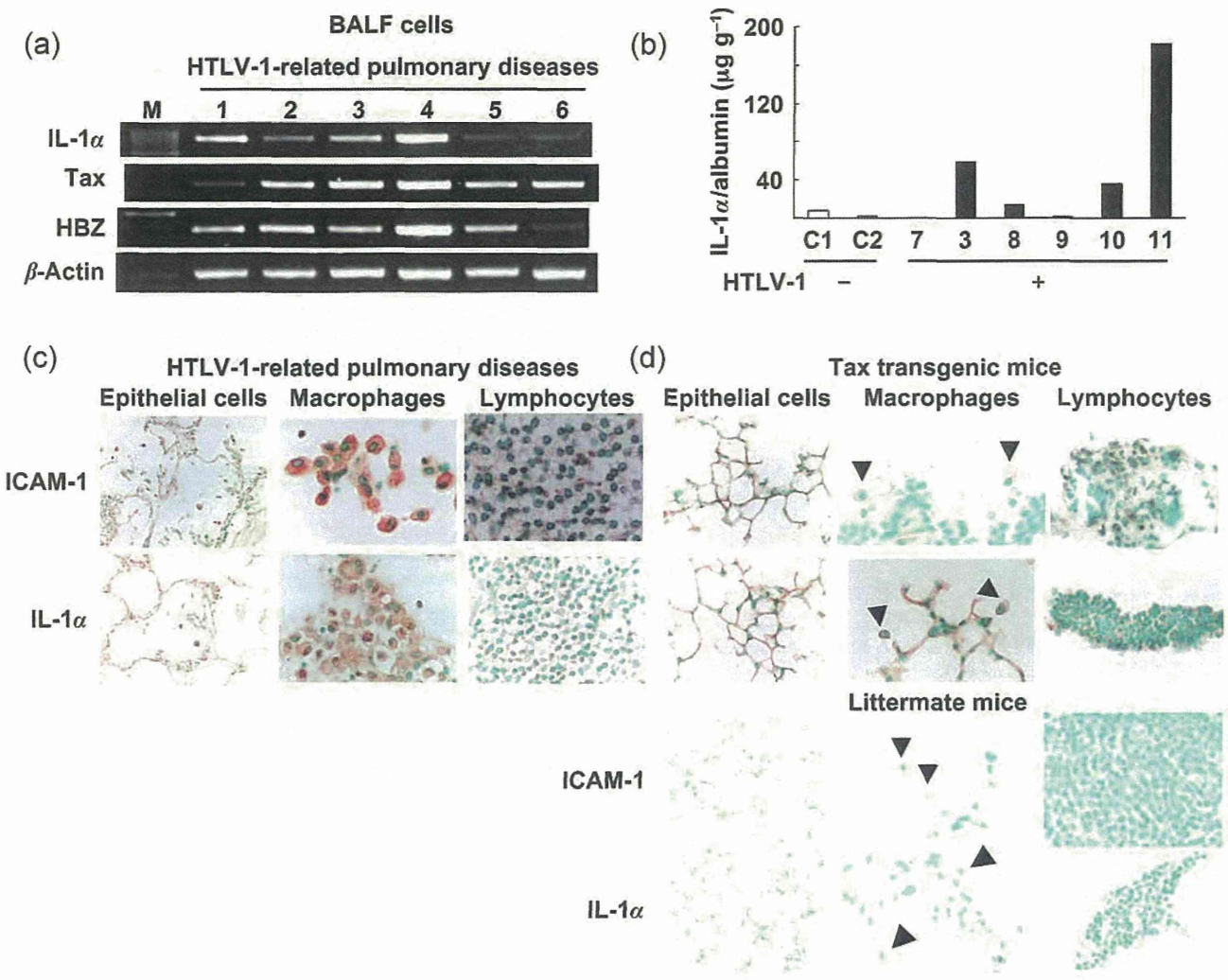

Fig. 7. Detection of IL-1α and ICAM-1 in the lungs of patients with HTLV-I-related pulmonary diseases and in Tax transgenic mice. (a) Detection of Tax, HBZ and IL-1α mRNAs in BALF cells obtained from six patients with HTLV-I-related pulmonary diseases. Human β-actin mRNA was used as a control. Lanes 1–6 denote patients 1–6. M, 100 bp ladder (Toyobo Life Science). (b) Concentration of IL-1α in BALF from patients with HTLV-I-related pulmonary diseases and non-infected controls. Case 3 is the same as patient 3 in (a). C1 and C2 were control subjects who were seronegative for HTLV-I. (c, d) Detection of IL-1α and ICAM-1 proteins by immunohistochemistry. In the lung tissues of patients with HTLV-I-related pulmonary diseases (c) and Tax transgenic mice (d), immunohistochemical staining showed a definite brownish staining for IL-1α and ICAM-1 proteins in the membrane and cytoplasm of epithelial cells, and in macrophages and infiltrated lymphocytes. Arrowheads indicate the surfaces of macrophages in lung tissues of mice. Cells were counterstained with methyl green.

mediates HTLV-I-infected T-cell adhesion to lung epithelial cells.

Taken together, the results of this study suggest that IL-1α may play a role in the upregulation of ICAM-1 expression on lung epithelial cells in patients with HTLV-I-associated pulmonary disorders. Such a process may be involved in the pathogenesis of HTLV-I-associated pulmonary disorders. To assess the possible action of IL-1α, further studies will be needed to investigate the effects of neutralizing antibody against IL-1α on the development of HTLV-I-related lung lesions.

In summary, the findings of the present study allow the construction of a hypothesis in which IL-1α, produced by HTLV-I-infected Tax^+ T cells, is involved in the upregulation of ICAM-1 on lung epithelial cells. We found upregulation of LFA-1, the counter-receptor for ICAM-1, in HTLV-I-infected Tax^+ T cells. The detection of Tax mRNA expression in BALF cells of patients with HTLV-I-related pulmonary diseases suggests that the lung is a preferential site for its expression. Indeed, Tax-expressing transgenic mice exhibited inflammatory changes with infiltration of lymphocytes in the lung (Miyazato *et al.*, 2000). Tax has been reported to upregulate IL-1α in T cells (Mori & Prager, 1996). Tax may be

involved in the development of lung inflammation caused by HTLV-I through the induction of local production of IL-1α. The adhesion of T cells to the lung epithelial cells may play an important role in the pathogenesis of HTLV-I-related pulmonary diseases.

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CASE REPORT

Epstein–Barr virus-associated primary central nervous system lymphoma in a patient with adult T-cell leukemia/lymphoma

Masahiro AMANO,[1] Kousuke MARUTSUKA,[2] Tetsuaki SUGIMOTO,[3] Tatemi TODAKA,[4] Mitsuru SETOYAMA[1]

[1]*Department of Dermatology, Faculty of Medicine,* [2]*Pathology Division, University of Miyazaki Hospital, Kiyotake,* [3]*Department of Neurosurgery, Wada Hospital, Hyuga, and* [4]*Department of Neurosurgery, Miyazaki Prefectural Nobeoka Hospital, Nobeoka, Miyazaki, Japan*

ABSTRACT

We present a case of Epstein–Barr virus (EBV)-associated primary central nervous system lymphoma (PCNSL) arising from a patient with cutaneous-type adult T-cell leukemia/lymphoma (ATLL). Extranodal sites affected by ATLL include the skin, lung, liver, gastrointestinal tract and central nervous system (CNS). CNS involvement usually occurs as an acute and lymphoma-type ATLL. PCNSL is a rare type of tumor and the vast majority of PCNSL are of B-cell lineage. Individuals with acquired, iatrogenic or congenital immunodeficiency are at increased risk of PCNSL, which is commonly associated with EBV. In our patient, the expression of latent infection membrane protein 1 (LMP1), EBV nuclear antigen 2 (EBNA2), and EBV-encoded small RNA (EBER) in tumor cells confirmed a type III latency of EBV infection. Human T-cell lymphotropic virus type I (HTLV-I) can induce immunodeficiency before the overt development of ATLL. The HTLV-I infection led to suppression of the immune system and the development of EBV-associated PCNSL. This is the first reported case of the clinicopathological features of EBV-associated PCNSL arising from a patient with ATLL.

Key words: adult T-cell leukemia/lymphoma, Epstein–Barr virus-associated primary central nervous system lymphoma, type III latency of Epstein–Barr virus infection.

INTRODUCTION

Adult T-cell leukemia/lymphoma (ATLL) was first noted as a new type of leukemia by Takatsuki *et al.*[1] ATLL is a malignant lymphoproliferative disorder caused by human T-cell lymphotropic virus type I (HTLV-I).[2–4] It is endemic in south-west Japan and the Caribbean basin.[5] There are several subtypes of HTLV-I-induced ATLL: acute, lymphoma, chronic and smoldering.[6] ATLL commonly involves the skin in 43–72% of patients.[7] We proposed a fifth category: cutaneous-type ATLL.[8] Extranodal sites of involvement include the skin, lung, liver, gastrointestinal tract and central nervous system (CNS).[9–11] CNS involvement, whilst well recognized, usually occurs with systemic conditions. Isolated initial CNS involvement is exceedingly rare, with only one reported case to date.[12] Primary CNS lymphoma (PCNSL) is a rare tumor accounting for less than 2% of all intracranial neoplasms.[13] The vast majority of PCNSL are of B-cell lineage and PCNSL of HTLV-I infection are associated with ATLL.[9]

Individuals with acquired, iatrogenic or congenital immunodeficiency are at increased risk of PCNSL,[14] which commonly presents as a monoclonal, B-cell neoplasm classified as either diffuse large-cell or

Correspondence: Mitsuru Setoyama, Department of Dermatology, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan. Email: mtrstym@fc.miyazaki-u.ac.jp
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