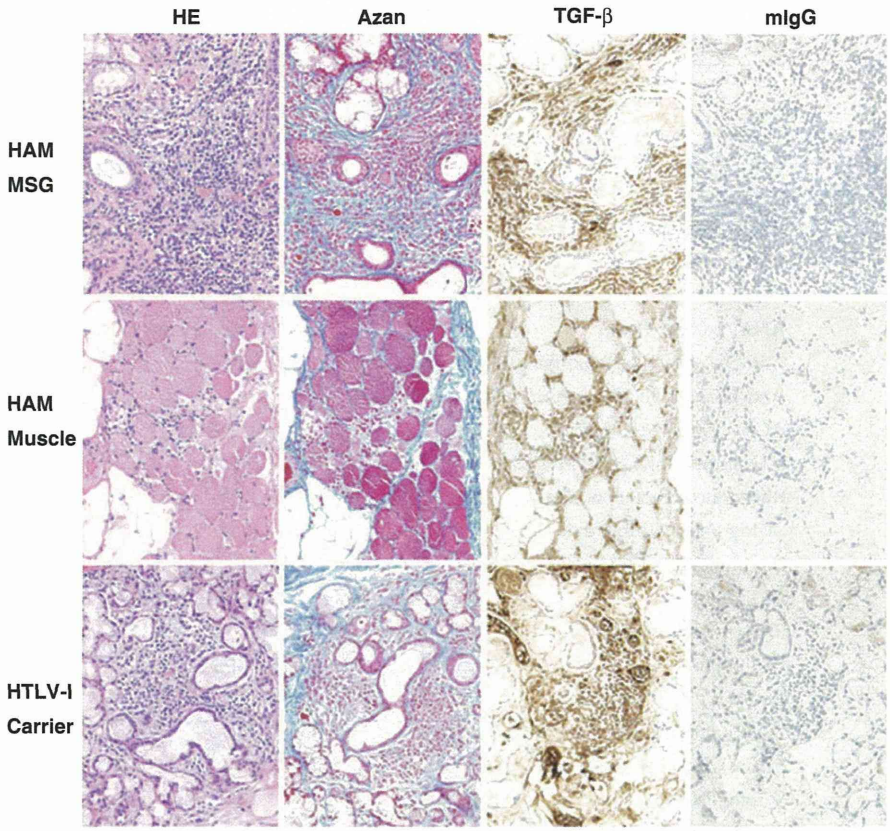

Fig. 1 Azocarmine with aniline blue (Azan)–Mallory staining and transforming growth factor beta (TGF-β) immunostaining in the labial salivary gland (LSG). Azan–Mallory staining and immunohistochemistry after epitope retrieval were performed for formalin-fixed, paraffin-embedded sections (3-μm thick) from the LSG using the Histofine Simple Stain Kit (Nichirei Co., Tokyo, Japan). The primary antibodies used for immunohistochemistry were TGF-β and mouse immunoglobulin (Ig)G1 (×200). Hematoxylin was used as a counterstain

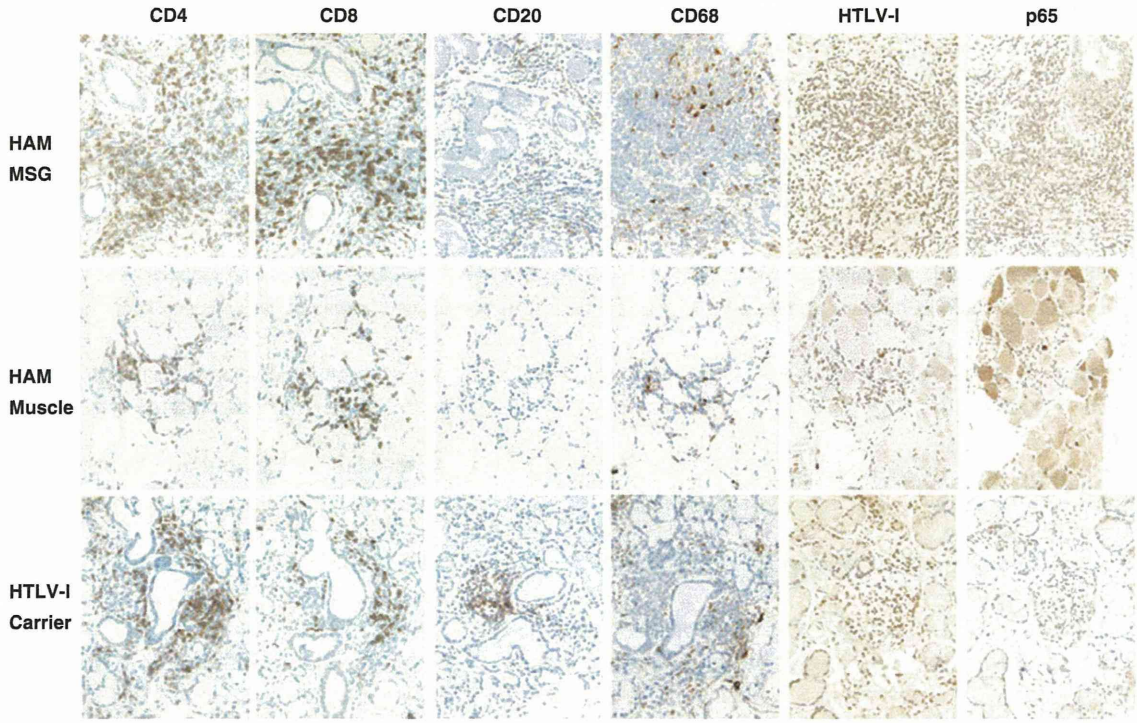

Fig. 2 Immunohistochemistry in the labial salivary gland (LSG). Immunohistochemistry after epitope retrieval was performed for formalin-fixed, paraffin-embedded sections (3-μm thick) from the LSG using the Histofine Simple Stain Kit (Nichirei Co., Tokyo, Japan). The primary antibodies used for immunohistochemistry were CD4, CD8, CD20, nuclear factor kappa B (NF-κB) (p65), and human T-cell leukemia virus type I (HTLV-I) [p19, p28, group-specific antigen (GAG)]. Lymph node from a patient with adult T-cell leukemia was used as a positive control for staining HTLV-I-related proteins (data not shown) (×200). Hematoxylin was used as a counterstain

and CD8+ lymphocytes were found in a muscle specimen from patient 2. It is interesting to note that HTLV-I-related proteins including p19, p28, and GAG were detected in the nuclei of a large percentage of infiltrating MNCs in LSGs and in the muscle specimen in patient 2, which was in accordance with the distribution of NF-κB p65.

TGF-β immunostaining in SS in the presence or absence of anti-HTLV-I antibody or ACA

We finally showed TGF-β immunostaining according to the presence of anti-HTLV-I antibody or ACA (Fig. 3). We performed these experiments in four patients each in three groups and show representative results (Fig. 3). In the HTLV-I-seropositive SS patients without ACA, TGF-β was dominantly found in vascular endothelial cells or fibrous tissues in LSG; however, the frequency of TGF-β+ cells (patients A–D in Fig. 3) appeared to be lower than the patients in cases 1 and 2 in Fig. 1. In the HTLV-I-seronegative SS patients with ACA, TGF-β was seen in infiltrating MNCs, vascular endothelial cells, and fibrous tissues in LSG. Then, in the HTLV-I-seropositive SS patients without ACA, TGF-β expression was similar to HTLV-I-seronegative SS patients with ACA (patients E–H in Fig. 3). In contrast, TGF-β expression was less in HTLV-I-seronegative patients without ACA (patients I, K, L) compared with other groups. In a HTLV-I-seronegative SS patient without ACA (as in patient J), TGF-β was not found in fibrous cells but in MNCs.

Discussion

Both HTLV-I and ACA are known to contribute to SS [1–8]; however, this coincidence of HTLV-I and ACA is supposed to occur with low frequency [8]. Our two cases presented here are rare but may illustrate the in vivo role of HTLV-I in patients with ACA-seropositive SS. Although both patients showed grade 3 MNC infiltration in LSGs, results from exocrine function tests, including Schirmer test and Saxon test in patient 1, were worse than those in patient 2. Except for the degree of MNC infiltration in LSGs, other factors such as aquaporin-5 distribution or type 3 muscarinic receptors [11, 12] might affect lacrimal and salivary secretion. With respect to MNC infiltration into the LSG, both cases showed similar findings. However, there were significant differences in fibrosis determined by Azan–Mallory staining and cytokine profiles.

As patient 2 was diagnosed with HAM, the HTLV-I viral load was high in comparison with patient 1, a finding that is consistent with previous reports [13]. Striking differences were observed in the Azan–Mallory staining findings; however, both patients showed high TGF-β expression in LSGs. TGF-β is a key cytokine for promoting the fibrotic process; thus, the prominent fibrosis of LSG is believed to be driven by TGF-β. Fibrosis was found in the LSG of both patients, which might be explained to some extent by the presence of ACA, as we previously reported [6]. However, a recent report found that HTLV-I basic-leucine zipper (bZIP) factor enhances TGF-β signaling through the p300 coactivator [14]. As strong expression of HTLV-I-related proteins was found in the LSG of patient 2, the TGF-β signaling pathways were suggested to be promoted in situ by HTLV-I, resulting in marked fibrosis. A similar phenomenon might occur in the muscle of patient 2, resulting in inflammatory myopathy. We previously reported that myopathy or uveitis was one characteristic of HTLV-I-seropositive SS patients [15]. With respect to a low level of IFN-γ, Santos et al. [16] demonstrated that administration of exogenous TGF-β induced a decrease of IFN-γ in cells from HTLV-I carriers, suggesting the possibility of the modulation of IFN-γ by TGF-β in HTLV-I-seropositive individuals. The high TNF-α level in patient 2 may also be driven by HTLV-I, as indicated for TGF-β.

To show the involvement of HTLV-I and ACA toward TGF-β expression, we examined TGF-β immunostaining for HTLV-I-seropositive patients without ACA, HTLV-I-seronegative patients with ACA, and HTLV-I-seronegative without ACA (Fig. 3). Although the precise quantitative analysis was not performed in this study, it may demonstrate that TGF-β expression in vascular endothelial cells and fibrous tissues of LSGs is more prominent in SS patients positive for both anti-HTLV-I antibody and ACA (two cases in Fig. 1) compared with SS patients positive for either one alone [two groups (patients A–H in Fig. 3)]. Accordingly, TGF-β expression in the above-mentioned sites was less in SS patients who were not positive for either anti-HTLV-I antibody or ACA (patients I–L in Fig. 3) in comparison with other groups. Therefore, we speculate that the synergistic effect of HTLV-I infection with ACA-carrying status induces the expression of TGF-β in LSGs, especially in vascular endothelial cells and fibrous tissue of SS patients (Fig. 4). However, we also found intense expression of TGF-β in MNCs even in HTLV-I-seronegative patients without ACA. As fibrous change determined by Azan–Mallory staining was not so significant in these patients, TGF-β in MNCs of LSGs may not be directly associated with the fibrotic process. In fact, TGF-β is known to be produced by CD4+ T lymphocytes [17] and influenced by other cytokines, such as IFN-γ [18]. Therefore, the two phenomena—Azan–Mallory-stain-proven fibrosis and TGF-β expression—should be carefully determined in patients with SS. Further studies with a larger number of participants and more definitive qualification approaches are necessary to prove our hypothesis.

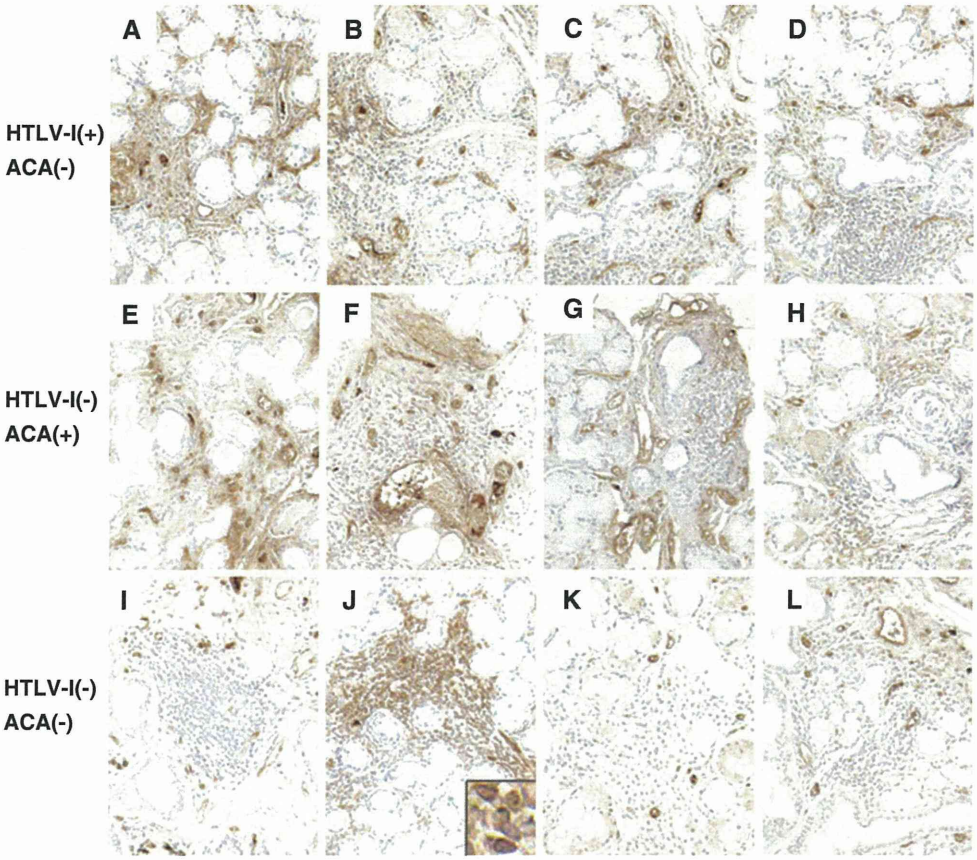

Fig. 3 Expression of transforming growth factor beta (TGF-β) in human T-cell leukemia virus type I (HTLV-I)-seropositive Sjögren's syndrome (SS) patients without anti-centromere-antibody (ACA), HTLV-I-seronegative SS patients with ACA, and HTLV-I-seronegative SS patients without ACA. Immunohistochemistry for TGF-β after epitope retrieval was performed for formalin-fixed, paraffin-embedded sections (3-μm thick) from the labial salivary gland (LSGs) using the Histofine Simple Stain Kit (Nichirei Co., Tokyo, Japan). Staining was performed for four HTLV-I-seropositive SS patients without ACA (patients A–D), four HTLV-I-seronegative SS patients with ACA (patients E–H), and four HTLV-I-seronegative SS patients without ACA (patients I–J). For patient J, TGF-β-positive MNCs are shown in the *inset* (×200). Hematoxylin was used as a counterstain

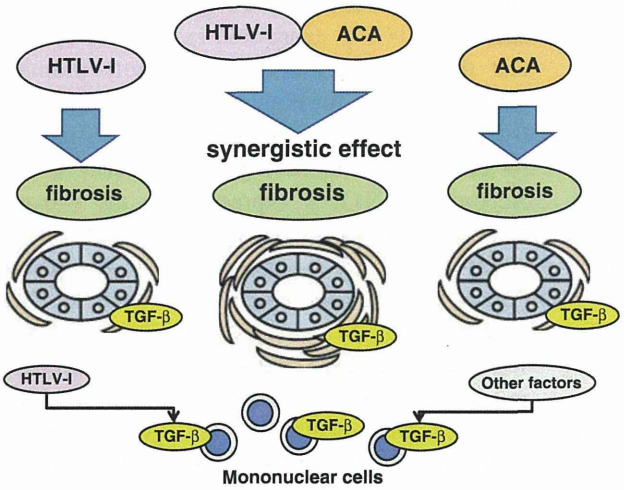

Fig. 4 Hypothesis for fibrotic alternation of salivary glands in Sjögren's syndrome (SS) patients through human T-cell leukemia virus type I (HTLV-I) infection and anti-centromere-antibody (ACA)-carrying status. From the results of the this study, HTLV-I- and ACA-carrying status induce fibrosis in labial salivary glands (LSGs). Furthermore, synergistic effects of HTLV-I infection with ACA-carrying status are assumed from the results of azocarmine with aniline blue (Azan)–Mallory staining. However, transforming growth factor beta (TGF-β), especially in mononuclear cells (MNCs), is also induced in HTLV-I infection and ACA-carrying status

In summary, we report two cases of ACA-seropositive SS found in HTLV-I-seropositive individuals and compared these patients with HTLV-I-seropositive SS patients without ACA, HTLV-I-seronegative SS patients with ACA, and HTLV-I-seronegative SS patients without ACA. The predominant characteristics were found in a patient with HAM, which was believed to have been caused by elevated HTLV-I viral load and subsequent cytokine production. Elements other than TGF-β are also suggestive of influencing fibrotic alternation of LSGs in patients with SS.

Conflict of interest None.

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特集

生物学的製剤の使用前と使用中における感染症（B型肝炎を含む）のスクリーニングとモニタリングをいかにおこなうか？

生物学的製剤使用前におけるHTLV-1のスクリーニングと使用中のモニタリング

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Key Words ■生物学的製剤 ■human T-cell leukemia virus type 1 ■adult T-cell leukemia ■HTLV-1-associated myelopathy

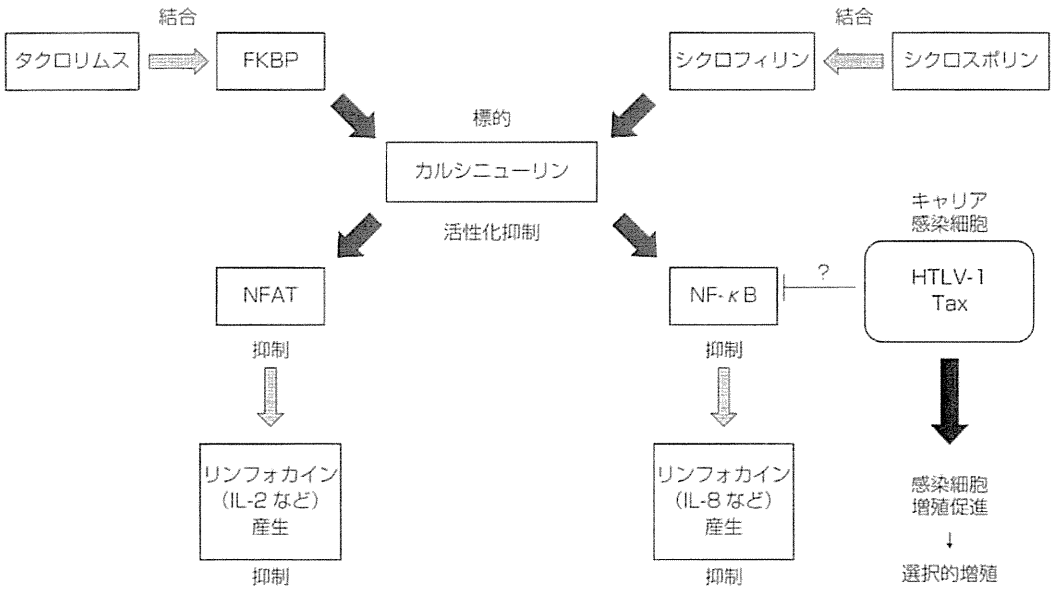
ヒトT細胞白血病ウイルス1型（HTLV-1）感染者での免疫抑制は成人T細胞白血病（ATL）/HTLV-1関連脊髄症（HAM）発症と関連すると考えられる．関節リウマチに対する生物学的製剤投与は現在ATL/HAM発症のリスク因子とはされていないものの，高度の免疫抑制をきたす場合があるので注意が必要である．生物学的製剤投与中におけるウイルス量のモニタリングなどはATL/HAM発症予測に有用である可能性がある．

はじめに

ヒトT細胞白血病ウイルス1型（human T-cell leukemia virus type 1：HTLV-1）はヒトTリンパ球へ感染し，プロウイルスとして感染細胞中に長期に潜伏するレトロウイルスの一種であり，成人T細胞白血病（adult T-cell leukemia：ATL）またHTLV-1関連脊髄症（HTLV-1-associated myelopathy：HAM）の原因となるウイルスとして広く認識されている．おもな感染経路は授乳による垂直感染と性交渉による水平感染である[1]．キャリアの多くが終生無症候である一方，経過中3～5%がATL，0.25～3%がHAMを発症する[2]．わが国では西日本とりわけ長崎，鹿児島，宮崎，沖縄といった地域が高浸淫地域として知られている．

1．リウマチ・膠原病患者におけるHTLV-1感染

リウマチ・膠原病の発症とHTLV-1感染との関連については以前から多くの研究がなされてきた．岩橋らが鹿児島県でおこなった調査では関節リウマチ（rheumatoid arthritis：RA）患者群での抗HTLV-1抗体陽性率は22.7%，全身性エリテマトーデス患者で25.7%と健常コントロール群（17.2%）と比較して高値であった[3]．当科の長崎県における研究では，RA[4]やシェーグレン症候群においては抗HTLV-1抗体陽性率が有意に高く，また，放射線影響研究所のコホート研究では，HTLV-1感染とシェーグレン症候群の関連性を報告した[5]．HTLV-1感染者においてリウマチ・膠原病が比較的高頻度に発症する原因は不明であるものの，活性化リンパ球からのサイトカイン産生増加が関与していると考えられている[6]．また混合性結合組織病（mixed connective tissue disease：MCTD）もHTLV-1感染との関連が考えられてお



図❶ タクロリムス・シクロスポリンの作用機序と HTLV-1 感染細胞
宮崎大学医学部内科学講座免疫感染病態学分野（膠原病感染症内科）ホームページ
（http://www.med.miyazaki-u.ac.jp/menekikansen/classroom/research.html）より引用

り，発症機序として U1-ribonucleoprotein と p30 gag protein とのアミノ酸配列における相同性が想定されている[7]．

2．HTLV-1 感染者に対する免疫抑制療法と ATL/HAM 発症との関連

HTLV-1 プロウイルス量の増加は ATL，HAM の発症リスクと関連していると考えられている[8][9]．RA および膠原病を発症した HTLV-1 感染者では，無症候性 HTLV-1 感染者とくらべてプロウイルス量が増加していたという報告もある[10]．

HTLV-1 感染者の免疫機能低下は ATL 発症と関連する可能性が示唆されている．移植領域における免疫抑制療法との関連として，生体肝移植後の拒絶反応抑制目的でタクロリムス（TAC）を投与されていた HTLV-1 キャリアが ATL を発症した例が報告されている[11][12]．移植領域で用いられる TAC，シクロスポリンはカルシニューリン阻害薬であり，nuclear factor of activated T-cells（NFAT），nuclear factor-kappa B（NF-κB）の活性変化が影響していると考えられる（**図❶**）．リウマチ・膠原病領域では，抗 HTLV-1 抗体陽性で間質性肺炎を合併した MCTD に対してプレドニゾロン治療を開始した後 ATL を発症した報告がある[7]．無症候性 HTLV-1 キャリアにおいては宿主免疫が ATL 発症を強力に抑制しているところ，免疫抑制療法の開始後免疫能の低下に伴いウイルス遺伝子の増殖抑制作用が失われ，ATL 発症に至ることが窺われる[13]．HTLV-1 感染者に対する免疫抑制療法中の ATL/HAM 発症については患者の原疾患，HTLV-1 ウイルス量や免疫抑制剤の種類，用量などさまざまな因子が関与していると考えられる．

つぎに，HTLV-1 感染患者に対する生物学的製剤投与について，現状ではどのようなことが明らかになっているであろうか．本稿執筆時点で上市されており，RA に対する適応を認められている生物学的製剤について，RA 治療として投与した際に ATL/HAM を発症した報告の有無について文献検索（PubMed，医学中央雑誌）をおこなったところ，現時点ではそのような報告を見出すことはできなかった．梅北らは HTLV-1 抗体陽性 RA 患者 2 例に対し生物学的製剤（1 例抗 TNFα 抗体，もう 1 例は可溶性 TNFα 受容体製剤）を投与し 6ヵ月後に評価をおこなった．投与した 2 例については HTLV-1 プ

ロウイルスコピー数の著変は認めなかったものの，RAに対する効果はno responseであった[14]．最近，われわれはスクリーニング検査で抗HTLV-1抗体陽性であったRA患者が，メトトレキサートおよびトシリズマブ投与中にATLを発症した例を経験したが[15]，これが本邦初の報告と考えられる．

3．HTLV-1のスクリーニングと使用中のモニタリングについて

通常おこなわれるHTLV-1感染のスクリーニングは血液検査による抗HTLV-1抗体の検出である．生物学的製剤投与前においても，まずは抗HTLV-1抗体測定でのスクリーニングが勧められる．抗HTLV-1抗体陽性である症例に対する生物学的製剤投与中はATL/HAMの症状発現に注意する必要がある．すなわち発熱，皮膚紅斑やリンパ節腫脹など身体所見の変化，血球計数や血清Ca値など検査所見の変化に留意して観察していく必要がある．

前述のとおりATL/HAMの発症には免疫抑制に伴うウイルス量増加が関与していると考えられているため，ウイルス量のモニタリングはATL/HAMの発症予測に有用と考えられる．現在厚生労働省研究班による免疫抑制療法中のHTLV-1ウイルス量とHTLV-1関連疾患発症との関連についての研究（班長 宮崎大学医学部膠原病感染症内科 岡山昭彦教授）が進行中である．この研究ではRAなどリウマチ・膠原病患者で免疫抑制剤投与による治療を継続されている患者を対象としてウイルス量を定期的に測定されており，免疫抑制療法中にHTLV-1キャリアにおけるウイルス量の動態が明らかになることが期待される．

おわりに

生物学的製剤投与中のATL/HAM発症について現時点では症例報告にとどまっており，明らかな頻度は不明である．しかしながら今後の生物学的製剤投与例増加に伴い，抗HTLV-1抗体陽性RA症例にも生物学的製剤が投与される機会も増えることが予想される．前述のとおりステロイド，免疫抑制剤また生物学的製剤を投与中のATL発症例は散見されるため，抗HTLV-1抗体陽性者に対してこれらの薬剤を投与する期間は注意深い観察を要する．抗HTLV-1抗体陽性RA患者に対する生物学的製剤投与開始後の変化，殊にプロウイルス量の変動とATL/HAM発症については引きつづき注視が必要と考えられる．

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シェーグレン症候群の環境要因：HTLV-Ⅰ

中村英樹* 高橋良子* 寶來吉朗* 川上 純*

シェーグレン症候群(SS)発症にかかわる環境因子の1つとしてウイルス感染が挙げられる．ヒトT細胞白血病ウイルスⅠ型(HTLV-Ⅰ)とSSとの関連性は，疫学的には知られている．その発症機序は不明な点が多いが，HTLV-Ⅰ関連脊髄症(HAM)を合併したSS小唾液腺は炎症の割に破壊が少なく，その一因として二次濾胞の出現頻度が少ないことが挙げられる．さらに二次濾胞形成に必要なケモカインCXCL13とその産生細胞である濾胞性樹状細胞も，HAMを合併したSSの小唾液腺ではみられないことも明らかとなってきた．実際，HTLV-Ⅰが唾液腺上皮細胞に感染しうるのか，あるいは液性因子を介した間接的な効果であるのかについて検討中であるが，現在までの結果を概説したい．

はじめに

シェーグレン症候群(Sjögren's syndrome：SS)では，病理学的に唾液腺，涙腺や炎症臓器へのCD4$^+$T細胞を中心とする単核球浸潤が観察される．SSの発症原因として女性ホルモンの関与，Fas/Fasリガンドを介した細胞死や，ヒトT細胞白血病ウイルスⅠ型(HTLV-Ⅰ)などのレトロウイルス，Epstein-Barr(EB)ウイルス，C型肝炎ウイルス感染などによるさまざまな環境因子が想定されている[1]．Greenら[2]は，1989年にHTLV-Ⅰの*tax*遺伝子を導入したマウスにおいて，SSに酷似した唾液腺炎および涙腺炎の発現を報告した．その5年後われわれは，抗HTLV-Ⅰ抗体陽性SS患者唾液中のIgA型抗HTLV-Ⅰ抗体とHTLV-Ⅰプロウイルスの存在より，これら患者には，HTLV-Ⅰ特異的な免疫反応が存在することを確認した[3]．また当科における検討で，HTLV-Ⅰ関連脊髄症(HTLV-Ⅰ associated myelopathy：HAM)において高率にSSを合併[4]することを示し，その後の疫学的検討[5]でも18名のSS患者中5名(27.8%)が抗HTLV-Ⅰ抗体陽性であり，834名の非SS群において70名(8.4%)が抗HTLV-Ⅰ抗体陽性であったことを示し，統計学的に有意にSSにおいて抗HTLV-Ⅰ抗体陽性率が高いことを示した．しかし，われわれのいくつかの検討において，抗HTLV-Ⅰ抗体陰性群と陽性群間では，乾燥症状や自己抗体の出現頻度には有意差がみられず，統計学的有意差を支持できるデータがなかった．その後の臨床病理学的検討で，徐々にこれらが明らかになりつつある．

〔キーワード〕
HTLV-Ⅰ
アポトーシス
二次濾胞
CXCL13

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Ⅰ．臨床的疫学的検討におけるSSとHTLV-Ⅰ感染の関係

当科における抗HTLV-Ⅰ抗体陽性SSの臨床的検討[6]では，抗HTLV-Ⅰ抗体陰性例に比較して，腺外症状が多いことが1つの特徴であった．すな

表 1. 抗 HTLV-Ⅰ抗体陰性 SS と陽性 SS の血清学的比較検討(Nakamura H *et al*, 2008[2]より改変引用)

	抗 HTLV-Ⅰ抗体		P 値
	陰性群	陽性群	
N(M/F)	37(3/34)	23(5/18)	
年齢	55.3±12.9	60.3±11.5	0.13*
抗核抗体	30/36(83.3%)	15/20(75.0%)	0.45**
抗 SS-A/Ro 抗体	17/37(45.9%)	9/23(39.1%)	0.60**
抗 SS-B/La 抗体	4/37(10.8%)	3/23(13.0%)	0.79**
IgG	2473.2±1268.5	2210.4±1144.4	0.45*

統計学的に抗 HTLV-Ⅰ抗体の有無で年齢，抗核抗体，自己抗体，IgG 値に有意差はみられなかった．
*Student *t*-test，**Chi-square test
P<0.05：有意

わち，筋症やぶどう膜炎あるいは反復する発熱が抗 HTLV-Ⅰ抗体陽性群で多くみられた．つぎに献血者を対象として抗 HTLV-Ⅰ抗体陽性率を検討したところ，27,284 人中 916 人(3.6%)が，抗 HTLV-Ⅰ抗体陽性であったのに対し，SS74 名中 17 名(23.0%)が抗 HTLV-Ⅰ抗体陽性であり，統計学的に有意であった[3]．一方，献血者と全身性エリテマトーデス患者における抗 HTLV-Ⅰ抗体陽性率には有意差はみられなかった．さらに，抗 HTLV-Ⅰ抗体陽性 SS では 71.4% (5/7 例)で IgA 型抗 HTLV-Ⅰ抗体を認め，HTLV-Ⅰと SS の関連を示す傍証と考えられた．しかし，抗 HTLV-Ⅰ抗体陰性 SS と陽性 SS で血清学的比較検討をくり返しおこなったが，抗核抗体，抗 SS-A/Ro 抗体，抗 SS-B/La 抗体，血清 IgG などの陽性率に有意差は認めなかった(**表 1**)．また，HAM20 名の検討[7]においては 13 名(65%)と高頻度に SS を合併することを示した．ただし，この時は 1993 年の European Community による分類基準を用いており，自覚症状の採用や自己抗体の有無などで厚生省改訂診断基準とは若干異なっている．2012 年に発表された米国リウマチ学会(American College of Rheumatology：ACR)の分類基準では，眼科所見・口唇生検・自己抗体の 3 項目のみが採用されており，今後これらの診断基準を比較した場合の HTLV-Ⅰ関連 SS の合併頻度の再評価が必要と思われる．

2．抗 HTLV-Ⅰ抗体陽性および陰性 SS の画像的・病理学的差異

前述の HAM 合併 SS 小唾液腺では，$CD4^+$ T 細胞が優位に浸潤しており浸潤自体は強いものの，抗 HTLV-Ⅰ抗体陰性 SS とは浸潤単核球フェノタイプに明らかな差は認めなかった[4]．また，唾液腺上皮アポトーシスにかかわる Fas や Fas リガンドの発現を抗 HTLV-Ⅰ抗体陽性および陰性 SS 小唾液腺組織について TUNEL 法にて比較検討した[8]が，これらの発現頻度や様式に有意差は認めなかった(**図 1**)．細胞死とその拮抗分子という観点から CD40/CD40 リガンドや Bcl-2 ファミリー蛋白[9]，X chromosome-linked inhibitor of apoptosis protein (XIAP)[10]および c-Jun N-terminal kinase(JNK)や p38 といった mitogen-activated protein kinase(MAPK)の発現[11]についても抗 HTLV-Ⅰ抗体陽性群と陰性群で比較したが有意な差異は観察されなかった．しかし，抗 HTLV-Ⅰ抗体陽性 SS 患者 23 例および陰性 SS 患者 37 例について，唾液腺破壊の程度と細胞浸潤のあいだに何らかの差がないか，唾液腺造影と口唇生検の結果を比較検討[12]した結果，はじめて

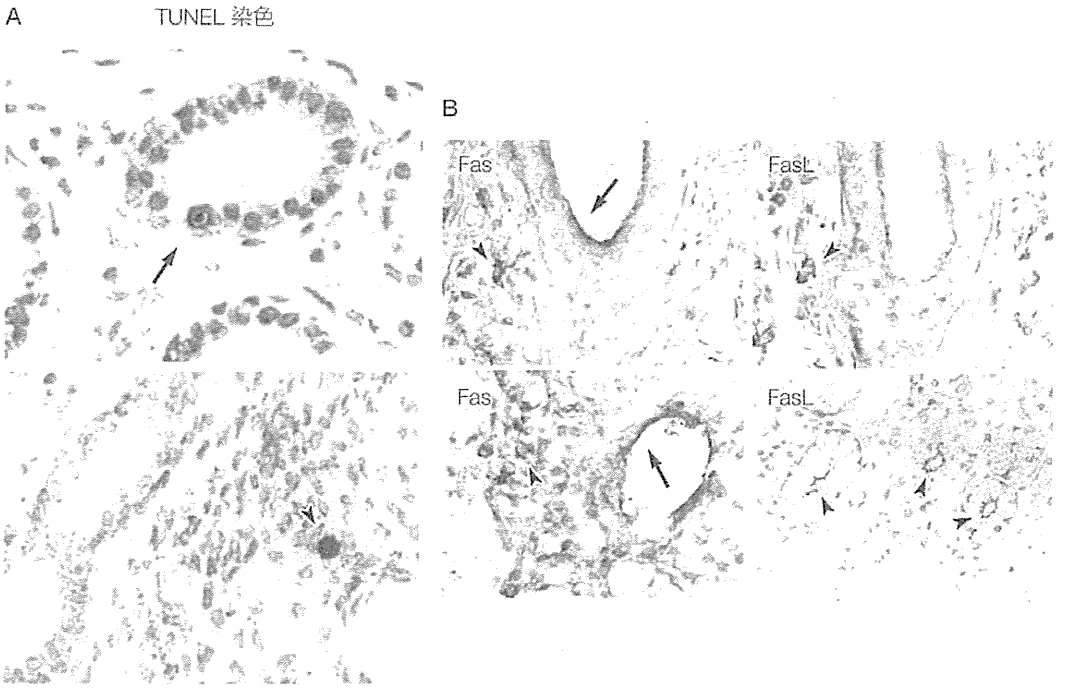

図 1．抗 HTLV-Ⅰ抗体陽性 SS 小唾液腺におけるアポトーシスと Fas/Fas リガンドの発現（Nakamura H *et al.* 1998[8] より改変引用）
A：TUNEL 染色により導管および浸潤単核球にアポトーシスを検出したが，抗 HTLV-Ⅰ抗体の有無で有意差はなかった．
B：免疫染色（DAB 発色）により Fas/FasL（ligand）の発現を導管内腔や浸潤細胞に認めた．

抗 HTLV-Ⅰ抗体の有無で差が観察された．すなわち，大きな有意差ではないものの，抗 HTLV-Ⅰ陽性 SS 群では，炎症細胞浸潤は観察される一方，唾液腺造影での破壊像が少ないことが明らかとなった．この理由として着目したのが，SS において報告が増えつつあった二次濾胞形成である[13][14]．すなわち，異所性二次濾胞陽性率は抗 HTLV-Ⅰ陰性 SS 32 例中 6 例（18.8%）に対し，抗 HTLV-Ⅰ陽性 SS 32 例中 1 例のみ（3.1%）であり有意差を認めた（$P=0.045$）[15]．また HAM 合併 SS 9 例では二次濾胞陽性例はみられなかった．また二次濾胞と同時に，二次濾胞形成に必要なケモカインであり，B 細胞分化にも必須である CXCL13 の発現も抗 HTLV-Ⅰ陽性 SS では観察されず，抗 HTLV-Ⅰ陽性 SS のはじめての病理学的特徴を捉えたものと考えられる（**図 2**）．他の HTLV-Ⅰ関連 SS の病理学的検討としては，抗 HTLV-Ⅰ抗体および抗セントロメア抗体陽性 SS における小唾液腺での transforming growth factor（TGF）-β 発現も検討[16]したが，これについては今後さらに線維化機序についても検討予定である．

3．HTLV-Ⅰの SS 唾液腺上皮細胞に対する影響

つぎに，SS 唾液腺における CXCL13 がその産生細胞と同定されている濾胞性樹状細胞（follicular dendritic cell：FDC）[17]から実際に分泌されているのか，また抗 HTLV-Ⅰ抗体の有無で差があるのかが課題となる．そこで，二次濾胞を有する抗 HTLV-Ⅰ抗体陰性 SS と HAM 合併 SS 小唾液腺で，FDC と CXCL13 の共発現を組織学的に検討した．この結果前者では，FDC と CXCL13 の共発現は明らかであったが，HAM 合併 SS ではいずれもみられず，抗 HTLV-Ⅰ抗体陽性群での

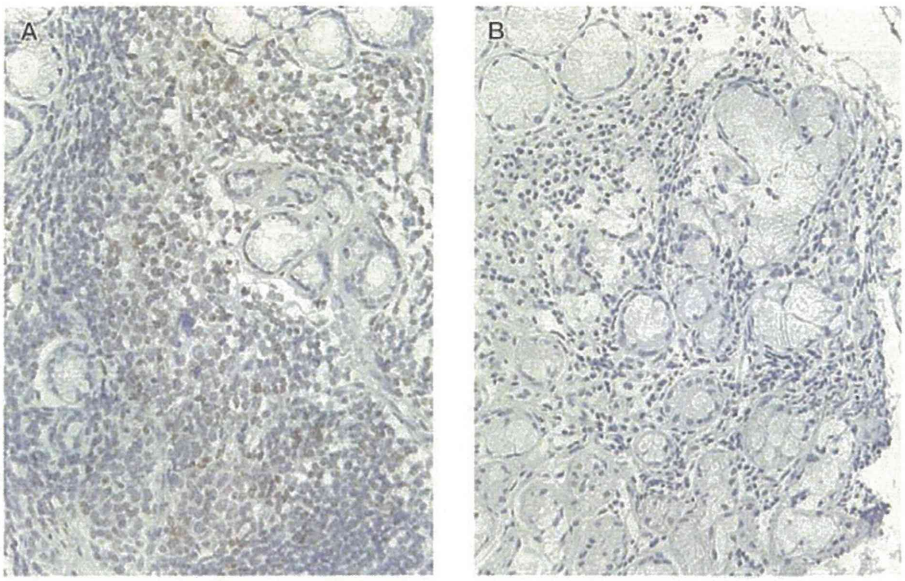

図 2. 二次濾胞形成と CXCL13 発現の差異(Nakamura H *et al*. 2009[15] より改変引用)
A：抗 HTLV-Ⅰ抗体陰性 SS 小唾液腺においてのみ二次濾胞が認められ，濾胞内には DAB 発色で陽性に染まる CXCL13 が観察された．
B：HAM 合併 SS 小唾液腺では二次濾胞形成は観察されず，CXCL13 発現もみられなかった．

組織学的な特徴の 1 つと考えられた．これらの結果を受けて，HTLV-Ⅰが唾液腺に対していかなる作用をしているか，あるいは HTLV-Ⅰが唾液腺上皮細胞に感染しうるのかが解明すべき点と思われる．これらを検討するため，HAM 患者髄液から樹立された HTLV-Ⅰ感染細胞株 HCT-5 と初代培養唾液腺細胞との共培養をおこない，培養上清中でのサイトカイン発現の変化を検討した．共培養 72 時間後の上清では，可溶性 intercellular adhesion molecule-1 (soluble ICAM-1：sICAM-1)，regulated upon activation, normal T-cell expressed, and secreted (RANTES) あるいは interferon γ-induced protein 10 kDa (IP-10：別名 CXCL10) などの明らかな発現増加がみられた(**図 3**)．sICAM-1 は細胞接着，RANTES は炎症における遊走能亢進に関与する分子であり，IP-10 は HAM において慢性炎症化に重要な分子と考えられている．これらの結果から，少なくとも小唾液腺浸潤単核球が接着・遊走しやすい環境が整っていることは推察される．さらに現在，共培養時の唾液腺上皮細胞を用いて，細胞死にかかわる分子発現についても検討中である．

おわりに

90 年代からの HTLV-Ⅰ感染と SS の関連を示す疫学的研究から SS 発症における HTLV-Ⅰの関与が示唆されていた．しかし，臨床データにおいてはこれまで抗 HTLV-Ⅰ抗体の有無で明らかな差異は認めていなかった．二次濾胞と CXCL13 発現の比較検討からこれまで明らかでなかった HTLV-Ⅰ関連 SS の病態を裏付けるデータの一部が次第に明らかとなりつつある．現在，HTLV-Ⅰと唾液腺の共培養の実験系から，蛋白レベルでの解析をおこなっているが，最終的には HTLV-Ⅰが唾液腺細胞に感染可能か否か，あるいは感染しなくても HTLV-Ⅰによる間接的な影響がどのように出るのかについて検討を進め，HTLV-Ⅰ関連 SS の発症機序を明らかにしていきたい．

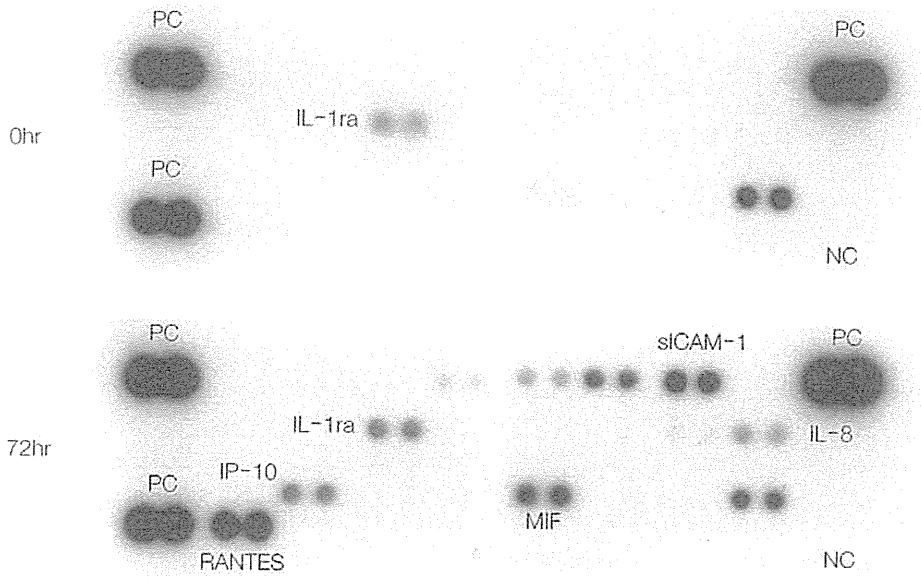

図 3. 培養唾液腺上皮細胞と HTLV-Ⅰ感染細胞株との共培養上清中のサイトカインアレイ
SS 患者より口唇生検で得られた小唾液腺の初代培養で得られた唾液腺上皮細胞と，HTLV-Ⅰ感染細胞株である HCT-5 との共培養をおこなった(長崎大学病院倫理委員会の承認済)．
共培養 72 時間の上清では，sICAM-1，RANTES や IP-10 などのサイトカインおよびケモカイン発現が明らかであった．
PC：positive control，NC：negative control

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Increased Expression of *HBZ* and *Foxp3* mRNA in Bronchoalveolar Lavage Cells Taken from Human T-lymphotropic Virus Type 1-associated Lung Disorder Patients

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Abstract

Objective Human T-lymphotropic virus type 1 (HTLV-I) causes adult T-cell leukemia/lymphoma (ATLL), and is associated with chronic inflammatory diseases, including inflammatory pulmonary diseases. *HTLV-I bZIP factor* (*HBZ*), which is expressed in all adult T-cell leukemia cells, plays a critical role in the development of lymphoma and systemic inflammation. HTLV-I is harbored by $CD4^+$ T cells that express *forkhead box P3* (*Foxp3*), and *HBZ* interacts with *Foxp3*. This study investigated the chest computed tomography (CT) findings and expression of *HBZ* and *Foxp3* in the bronchoalveolar lavage (BAL) cells from patients with HTLV-I-associated lung disorders.

Methods CT scans obtained from 37 patients (10 men and 27 women, aged 37-77 years) with HTLV-I-associated lung disorders were retrospectively evaluated. The expression levels of *HBZ* and *Foxp3* mRNA in BAL cells and the levels of inflammatory cytokines in the BAL fluid (BALF) from patients were compared with those in control subjects.

Results CT scans frequently revealed a diffuse panbronchiolitis (DPB)-like pattern, along with a nonspecific interstitial pneumonia (NSIP) pattern. An analysis of the BALF revealed lymphocytosis and increased expression of *HBZ* mRNA in patients with HTLV-I-associated lung disorders. The expression of *Foxp3* mRNA positively correlated with the percentages of lymphocytes present in the BALF. The inflammatory cytokine and IL-10 levels were significantly increased in the BALF from patients with HTLV-I-associated lung disorders.

Conclusion The NSIP pattern may be a manifestation of pulmonary involvement in HTLV-I-infected patients, as is the DPB-like pattern. *HBZ* and *Foxp3* likely have a role in the development of lung inflammation.

Key words: bronchoalveolar lavage (BAL), *forkhead box P3* (*Foxp3*), *HTLV-I bZIP factor* (*HBZ*), human T-lymphotropic virus type 1 (HTLV-I), nonspecific interstitial pneumonia (NSIP)

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Introduction

Human T-lymphotropic virus type 1 (HTLV-I) is known to be the causative agent of adult T-cell leukemia/lymphoma (ATLL), and is also associated with chronic inflammatory diseases, such as HTLV-I-associated myelopathy (HAM)/tropical spastic paraparesis (TSP), HTLV-I-associated uveitis, arthritis and Sjögren's syndrome. Pulmonary involvement has also been reported in patients infected with HTLV-

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Table 1. Characteristics of HTLV-I-infected Patients and Subsequent BALF Analysis

					Bronchoalveolar lavage fluid									
Case.no	Chest	Sex/Age	HTLV-1ab	Smoking(B.I)	Total Cells	AM(%)	Ly(%)	Neu(%)	Eo(%)	CD4(%)	CD8(%)	CD3(%)	CD3/25(%)	CD4/8
1	localized GGO	F/71	4096	0	1.05	89.6	6.4	3.4	0.6	14	8.21	22.2	22.2/2.0	1.7
2	diffuse GGO + centrilobular nodules	F/37	4096	40	4.8	27.9	64.1	5.3	2.6	53.9	33.9	89.3	89.5/2.7	1.6
3	coin lesions	F/60	8192	N/A	3.92	46.7	26.3	24.1	2.9	NT	NT	NT	NT	NT
4	localized consolidation	F/58	+	0	18.6	19.7	9.5	66.8	3.9	12.5	3.9	35.3	35.3/1.9	3.2
5	UIP	M/53	+	+	3.8	35.1	44	18.8	2.2	28.9	45.5	76.6	NT	0.6
6	coin lesions	F/62	4096	N/A	0.86	78.6	21.1	0.3	0	63.7	28.2	87.7	NT	2.3
7	coin lesions	F/62	+	0	1.05	62.5	36	1.4	0.1	51.1	25.6	78	NT	2
8	OP	M/60	4096	1000	2.59	50.7	44	0.5	4	11.3	3.2	NT	NT	3.5
9	NSIP pattern	F/59	4096	0	12.7	55.5	44.5	0	0	16.6	50.2	87.4	88.5/10.2	0.3
10	NSIP pattern	F/57	4096	450	4.8	99	0.6	0.2	0.2	NT	NT	NT	NT	NT
11	NSIP pattern	F/65	>8192	0	3.56	71.4	27.9	0.32	0.32	69.2	10	73.5	73.5/60.3	6.9
12	NSIP pattern	F/74	256	N/A	0.86	84.3	15.1	0.64	0	37.8	22.5	60.5	62.2/4.2	1.7
13	NSIP pattern	F/63	4096	0	1.9	93.8	4.8	1.4	0	41	72.6	75.2	80.1/1.5	0.6
14	NSIP pattern	M/60	4096	640	3.14	52.8	44.8	2.1	0.3	35.4	9.3	40.3	40.3/1.2	3.8
15	NSIP pattern	F/63	>8192	0	1.45	72.2	24	2.4	1.2	4.3	2.3	9	9.1/3.4	1.9
16	NSIP pattern	M/63	+	1640	6.94	48.3	46	3.8	1.9	64.05	16.8	83	83.0/7.3	3.8
17	NSIP pattern	F/65	256	0	2.27	49.6	42.9	6.8	0.8	84.9	9.65	95.7	96.1/1.2	8.8
18	NSIP pattern	F/64	+	0	6.9	76.5	17	5.3	1.1	0.2	6.9	11.6	11.6/0.8	0
19	NSIP pattern	F/75	+	0	2.5	67.7	18.3	13.4	0.57	22.6	14.3	36.9	37.0/4.13	1.6
20	DPB-like pattern	F/53	4096	0	1.87	99.4	0.4	0.2	0	40.7	45.9	75.8	NT	0.9
21	DPB-like pattern	M/77	>8192	>400	2.23	70.2	18.5	9.8	1.5	NT	NT	NT	NT	NT
22	DPB-like pattern	M/59	4096	0	20.3	28.3	43.2	0	10.9	9	89.6	97.7	NT	0.1
23	DPB-like pattern	F/61	4096	0	0.78	83.6	13.9	1.9	0.5	NT	NT	NT	NT	NT
24	DPB-like pattern	F/73	+	0	1.15	53.1	44.6	2.1	0.1	45.6	16.8	83.4	83.1/3.6	2.7
25	DPB-like pattern	F/69	+	0	5.38	38	52.6	8.5	0.9	15.1	28.9	42.3	42.3/2.49	0.5
26	DPB-like pattern	F/74	4096	0	0.9	67.8	18.9	13.1	0	2.4	24.8	NT	NT	0.1
27	DPB-like pattern	M/45	+	+	2.29	61.2	15.9	18.3	4.5	61.7	29.8	90.5	90.5/11.1	2
28	DPB-like pattern	M/76	4096	100	5.65	50	19	30	1	6.4	23.5	55.6	54.5/3.3	0.3
29	DPB-like pattern	F/61	4096	400	5.5	53.1	15	30.2	1.7	4.5	6.4	4.7	3.7/3.0	0.7
30	DPB-like pattern	F/69	16	0	1.25	58	8.9	32.5	0.5	35.2	30	61.5	61.8/17.5	1.2
31	DPB-like pattern	F/33	4096	0	8.9	26	35.6	38.3	0	1.1	5.2	7	NT	0.2
32	DPB-like pattern	F/67	1024	0	28.8	24.3	31.8	42.9	0.9	3.1	5.4	35.3	37.1/0.3	0.6
33	DPB-like pattern	F/65	4096	+	6.2	28.1	11.3	59.5	0.9	11.8	14.6	41.3	39.3/1.0	0.8
34	DPB-like pattern	F/52	8192	N/A	6.5	15	8.5	75.3	1.2	NT	NT	NT	NT	NT
35	DPB-like pattern	M/77	8192	1080	6.88	15.6	3.9	78.6	1.8	16.6	28.2	58.3	58.4/22.5	0.6
36	DPB-like pattern	F/70	1024	0	17.8	2.8	0.2	96.7	0.2	NT	NT		NT	NT
37	normal CT findings	F/68	+	0	2.4	68.1	30.2	0.7	1.1	NT	NT	NT	NT	NT
38	old inflammatory change	M/52	-	+	1.4	93.3	6.1	0.6	0	NT	NT	NT	NT	NT
39	round atelectasis	M/56	-	0	NT	88.7	9.8	1.5	0	NT	NT	NT	NT	NT
40	normal CT findings	F/15	-	0	0.78	90.5	5.6	0	3.9	14.1	8.78	17.9	18/1.5	1.6
41	normal CT findings	M/69	-	+	0.4	86.4	11.8	0.9	0.9	NT	NT	NT	NT	NT
42	normal CT findings	F/66	-	0	0.8	91.8	5.3	2.6	0.3	45.9	23.1	69.2	69.5/6.3	2
43	normal CT findings	F/50	-	0	0.83	84.3	8.3	7.4	0	30.7	27.4	70.8	70.8/8.3	1.1
44	normal CT findings	F/57	-	0	0.47	88	10.9	0.9	0.2	35.2	32.4	72.8	72.7/9.6	1.1
45	normal CT findings	M/32	-	40	0.6	86.5	13.1	0.2	0.2	43.9	18.2	71.4	NT	2.4

NT: not tested, N/A: not available, B.I.: Brinkman Index

I in the carrier state, such as in those with HTLV-I-associated inflammatory diseases, including HAM/TSP and uveitis (1, 2). It has been reported that there is an elevation in the levels of soluble interleukin-2 receptors in the bronchoalveolar lavage fluid (BALF) (2), along with increased expression of mRNA transcripts for inflammatory cytokines and chemokines in the bronchoalveolar lavage (BAL) cells of patients with HTLV-I-associated lung disorders (3, 4). The expression of some cytokines and *tax* (a factor implicated in HTLV-1 pathogenesis) mRNA expression correlated with the proportion of lymphocytes in the BALF (4). These data suggest that HTLV-I infection induces pulmonary inflammation, at least partially *via* the local production of inflammatory cytokines.

The manifestations of these inflammatory pulmonary involved in HTLV-I-infected patients vary widely, and include bronchiolitis, alveolitis, diffuse panbronchiolitis (DPB) and interstitial pneumonia (5-9). We recently reported on four HTLV-I-infected patients with histology corresponding to nonspecific interstitial pneumonia (NSIP) (10). NSIP is one of the subtypes of idiopathic interstitial pneumonia, which was first described by Katzenstein and Fiorelli (11), and is a

Table 2. Chest Radiographic Findings of HTLV-I Carriers

Chest radiographic findings (n, %)		Male/Female	Age (years)	CD/HAM/uveitis
DPB-like pattern	(17, 45.9)	5/12	67 ± 11.8	1/2/1
NSIP pattern	(11, 29.7)	2/9	63 ± 5.3	2/0/1
Others	(8, 21.6)	3/5	60 ± 9.2	1/0/0
Normal CT findings	(1, 2.7)	0/1	68	0/0/0
Total		10/27	63 ± 9.9	4/2/2

Others: coin lesions (n = 3); localized consolidation (n = 1); organizing pneumonia (n = 1); usual interstitial pneumonia (n = 1); diffuse GGO and centrilobular nodules (n=1); localized GGO (n=1).
CD: collagen disease, HAM: HTLV-I-associated myelopathy

histopathological pattern that is often seen in patients with immunodeficiency, following exposure to certain drugs or environments and in those with some connective tissue diseases. Using chest computed tomography (CT), the most common findings of NSIP are lower lobes with peripherally predominant ground-glass opacity and reticular abnormalities, traction bronchiectasis and lower lobe volume loss without honeycomb formation (12, 13). NSIP has rarely been reported among patients with HTLV-I-associated pulmonary involvement.

The proviral form of HTLV-I contains a unique region known as pX (14, 15). The pX region is located between the *env* gene and the 3' long terminal repeat (LTR) that encodes viral accessory genes, including *tax* and *HTLV-1 bZIP factor* (*HBZ*) (16), which are implicated in viral infectivity and the proliferation of infected cells (17-19). It is well known that *tax* activates transcriptional factors and plays a critical role in HTLV-I pathogenesis. However, *tax* transcripts are detected in only approximately -40% of adult T-cell leukemia (ATL) cases (15).

It has been shown that *HBZ* is constitutively expressed in both HTLV-I-infected cells and ATL cells (17). The transgenic expression of *HBZ* results in the development of T-cell lymphomas and systemic inflammatory diseases, including lung lesions in mice (20). Furthermore, the expression of *HBZ* correlates with the levels of inflammatory markers and the disease severity in HAM/TSP patients (21, 22). It has also been reported that ATL cells strongly express Foxp3; a marker specific for $CD4^+CD25^+$ regulatory T-cells (23-25). *HBZ* has been demonstrated to interact with *Foxp3*, and therefore, *tax* may be critical for disease initiation, while *HBZ* may promote disease progression. We have hypothesized that *tax* and *HBZ* both play critical roles in the pulmonary involvement of HTLV-I infection.

In this study, we investigated whether the NSIP pattern could be a manifestation of the pulmonary involvement in HTLV-I-infected patients. We evaluated the expression levels of *tax* and *HBZ* mRNA transcripts in BAL cells, and the levels of inflammatory cytokines in the BALF.

Materials and Methods

Subjects

Our study was reviewed and approved by the ethics review board of the University of the Ryukyus. The subjects investigated in our study were 37 HTLV-I carriers (10 men and 27 women, aged 37-77 years) who had lung disorders or respiratory symptoms, and visited the pulmonary division of the University of the Ryukyus Hospital from 1993 to 2010. The selection criteria for inclusion in the study were as follows: 1) chest CT scans were obtained, 2) BAL was performed and BALF was obtained and 3) there was no concurrent ATLL or development of ATLL. ATLL cells or other malignant cells were not detected in the BALF cytology or specimens obtained from the transbronchial lung biopsies in any cases in this study. We also excluded the presence of other infections, malignancy or other pulmonary diseases. HTLV-I seropositivity was determined using the particle agglutination method (New Seroclit-anti-HTLV-1; Sanko, Tokyo, Japan). Eight control subjects were also included who were seronegative for HTLV-1 (four men and four women, aged 15-69 years). The CT findings and the results of the BAL analyses are shown in Table 1.

Case 38 had no symptoms, and bronchoscopy was performed to investigate a linear band in the lower lobe of left lung. It revealed old inflammatory changes. Case 39 had no symptoms, and bronchoscopy was performed to investigate a lung tumor, and revealed round atelectasis. Six of the eight control patients had normal chest radiographic findings. Cases 40-42 had uveitis, and bronchoscopy was performed to exclude sarcoidosis. Bronchoscopy was performed to investigate chronic cough in the remaining three patients. They did not have abnormal findings in the BALF analysis and there were no signs of malignancy or active inflammation. We considered these patients to be control subjects.

Chest CT findings

Chest CT examinations were performed with a GE Light Speed scanner (GE medical systems, WI) or an Aquillion

CT scanner (Toshiba Medical Systems, Tochigi, Japan). Patients diagnosed with concurrent infectious diseases by serological tests, and/or based on the clinical and pathological findings were excluded from this study. Three Japanese board-certified pulmonologists retrospectively interpreted the chest CT scans. The chest CT images were assessed with respect to the radiological patterns. NSIP patterns were categorized according to the ground-glass opacity and/or homogeneity of the reticular pattern, with neither consolidation nor honeycombing. DPB-like patterns were defined as small centrilobular nodules with branching linear opacities, with a tree-in-bud appearance. Other abnormalities, including consolidation and honeycombing, were considered to be part of the "other" group.

BAL and cell preparation

BAL was performed as described previously (4). Under local anesthesia, a fiberscope was inserted into the middle lobe, and 150 mL of sterile saline solution in three 50-mL aliquots, was instilled. The fluid was recovered by gentle aspiration into a sterile syringe, and was passed through two gauze sheets. The BALF was transported to a laboratory at 4℃ within 30 min. The BAL cells were pelleted by centrifugation (1,400 × g, 10 min, 4℃), and the supernatant was collected and stored at -80℃ for further experiments. After being washed twice with calcium- and magnesium-free phosphate-buffered saline (PBS) supplemented with 1% heat-inactivated fetal calf serum (FCS), the total cells were counted using a hemocytometer. An aliquot was adjusted to 1×10^6 cells/mL, and the proportion of $CD4^+$, $CD8^+$ and $CD25^+$ cells were analyzed by flow cytometry (EPICS XL System II; Beckman Coulter, Fullerton, CA) after staining with a fluorescein isothiocyanate (FITC)-conjugated anti-CD4 or anti-CD8 monoclonal antibody (mAbs) (DAKO, Glostrup, Denmark) or a phycoerythrin (PE)-conjugated anti-CD25 mAb (DAKO). The remaining cells from the BALF were lysed in 1 mL of Isogen (Nippon Gene, Tokyo, Japan) and stored at -80℃ until required for subsequent analyses.

RNA isolation and quantitative PCR (qPCR) assays

To investigate the association of HTLV-1 infection with pulmonary involvement, we examined the *tax*, *HBZ* and *Foxp3* mRNA expression levels in the BAL cells from seven patients with a DPB-like pattern (cases 23, 25, 27, 30, 34, 35 and 40, Table 1), seven NSIP-patterned patients (cases 11, 12, 14-16, 18 and 19, Table 1) and three control subjects (cases 40, 42 and 43, Table 1). Total RNA was extracted from the BAL cells using Isogen (Nippon Gene; Tokyo, Japan), and reverse transcription was conducted using 1 μg of sample RNA with a high capacity reverse transcription kit (Applied Biosystems, CA, USA), following the manufacturer's instructions. All qPCR assays were performed using the ABI Prism 7000 sequence detection system. The primer sequences for *tax* mRNA transcript detection were 5'-ATC CCG TGG AGA CTC CTC AA-3' and 5'-CCA AAC ACG TAG ACT GGG TAT CC-3, along with a specific probe (5'-TCC AAC ACC ATG GCC CAC TTC CC-3') (26). The sequences of the primers used for spliced *HBZ* mRNA transcript detection were 5'-AGA ACG CGA CTC AAC CGG-3' and 5'-TGA CAC AGG CAA GCA TCG A-3'; and the probe sequence was 5'-TGG ATG GCG GCC TCA GGG CT-3' (21). For the detection of *Foxp3* mRNA transcripts, an established Taqman assay was used (Catalog number 4331182; Applied Biosystems, CA). For internal calibration of the qPCR assays, we used primers and probes specific for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Applied Biosystems, CA).

Determination of the cytokine levels in the BALF

To explore the possible mechanisms underlying the lung involvement of HTLV-1 infection, we examined the cytokine levels in the BALF from 27 HTLV-1 carriers (cases 1-5, 9, 11-14, 15-23, 25-28, 30 and 34-36, Table 1) and five control subjects (cases 38 and 40-43, Table 1). The BALF supernatant was concentrated using Centricon-Plus centrifugal filters (Centricon-Plus, Millipore, MA). We measured the cytokine levels in the BALF samples using the human cytokine group I multiplex cytokine assay (17-Plex; Bio-Plex, BioRad, Hercules, CA) and normalized the levels to those of albumin in the BALF. The normalized values were reported as the ratios of the cytokine concentrations in concentrated supernatants of the BALF samples to the albumin concentration in the concentrated BALF supernatants.

Statistical analysis

The data are expressed as the means ± standard deviation. Statistical comparisons were conducted using Student's *t*-test for the BAL analyses, and the Mann-Whitney U-test for the qPCR results. Pearson's correlation coefficient was used to evaluate the level of correlation. A p-value <0.05 was considered to be significant.

Results

Radiographic findings in patients with HTLV-1-associated lung disorders

Of the 645 patients who underwent BAL, 14.4% (93/645) were HTLV-I-positive. Based on the chest CT findings, bronchiectasis (n=19, 51.3%) was the most frequently observed abnormality. Centrilobular nodules (n=17, 45.9%), ground-glass opacities (n=16, 43.2%), thickening of the bronchovascular bundles (n=13, 35.1%) and reticular shadows (n=9, 24.3%) were also frequently observed. Areas of consolidation were observed in two patients (5.4%) and honeycombing was seen in one patient. The combination of bronchiectasis and centrilobular nodules (n=11, 29.7%), which were frequently observed in the DPB pattern, was the most frequently seen combination, followed by ground-glass opacities and reticular shadows (n=8, 21%). A typical image of a DPB-like pattern is shown in Fig. 1A (case 31, Ta-

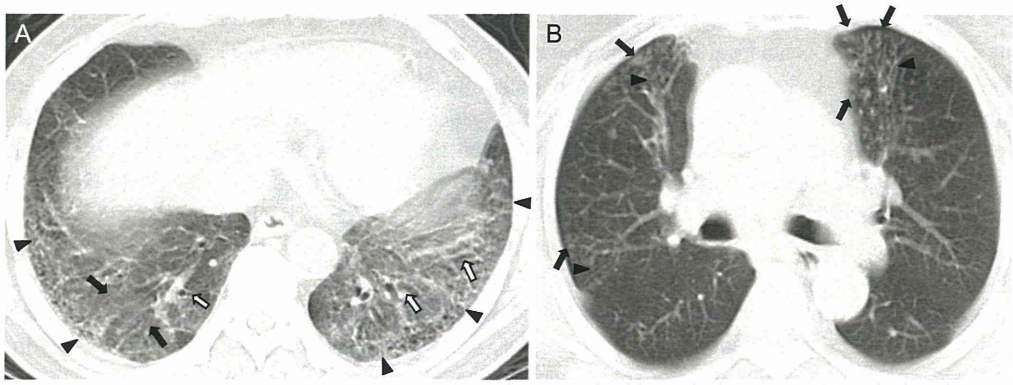

Figure 1. **(A) A transverse CT scan from a 60-year-old man with a categorized NSIP pattern showing peripheral ground-glass opacity (black arrow), a reticular shadow (black arrowhead) and traction bronchiectasis (white arrow). (B) A transverse CT scan from a 71-year-old woman with a categorized DPB pattern showing centrilobular nodules (black arrow) and dilated proximal bronchi (black arrowhead).**

Table 3. **Total and Differential Cell Counts in BALFs**

	Total Cells ($\times 10^5$/mL)	AM (%)	Ly (%)	Neu (%)	Eo (%)	CD4 (%)	CD8 (%)	CD3 (%)	CD3/25 (%)	CD 4/8
HTLV-1 carriers	3.56**	53.1	19**	5.3**	0.9	19.6	19.6	61	3.15	1.4
n=37	(0.78-28.8)	(2.8-99.4)	(0.2-64.1)	(0-96.7)	(0-10.9)	(0.2-84.9)	(2.3-89.6)	(4.7-97.7)	(0.3-60.3)	(0-8.8)
NSIP	3.14	71.4	24**	2.1	0.32	36.6	12.1	67	3.7	1.8
n=11	(0.86-6.9)	(48.3-99)	(0.6-46)	(0-13.4)	(0-1.9)	(0.2-84.9)	(6.9-72.6)	(9-95.7)	(0.8-60.3)	(0-8.8)
DPB	5.5**	50	15.9*	30**	0.9	11.8	24.8	56.9	3.3	0.6
n=17	(0.78-28.8)	(2.8-99.4)	(0.4-52.6)	(0.2-96.7)	(0-10.9)	(1.1-61.7)	(5.2-89.6)	(4.7-97.7)	(1-22.5)	(0.1-2.7)
normal CT findings n=1	2.4	68.1	30.2	0.7	1.1	NT	NT	NT	NT	NT
Control	0.78	88.3	9.05	0.9	0.2	35.2	23.1	70.8	7.3	1.6
n=8	(0.4-1.4)	(84.3-93.3)	(5.3-13.1)	(0-7.4)	(0-3.9)	(14.1-45.9)	(8.8-32.4)	(17.9-72.8)	(1.5-9.6)	(1.1-2.4)

*$p < 0.05$, **$p < 0.01$ compared with control subjects (*t*-test).

Values are presented as medians (range).

ble 1), and a typical image of the NSIP pattern is shown in Fig. 1B (case 17, Table 1). Of the HTLV-I carriers, a DPB-like pattern was observed in 45.9% (17/37) of the patients, and a NSIP pattern was seen in 29.7% of the patients (11/37). One patient (case 37, Table 1) had normal CT findings. Coin lesions, consolidations, diffuse ground glass opacity and centrilobular nodules and usual interstitial pneumonia were observed in the remaining patients (Table 2).

BAL analysis

The anti-HTLV-I antibody titers were consistently high in the HTLV-1 carriers (Table 1). A summary of the results of the BAL analyses for the 37 HTLV-I carriers is presented in Table 3. The total cell numbers and the percentages of lymphocytes in the BALF samples were significantly increased in HTLV-I carriers compared with the control subjects (3.56×10^5/mL and 0.78×10^5/mL, 19% and 9.05%, respectively, $p<0.01$). The percentages of neutrophils in the BALF samples were also significantly increased in patients with a DPB-like pattern compared with patients exhibiting NSIP and control subjects (30%, 2.1% and 0.9%, respectively, $p<0.01$). There were no significant differences in the percentages of T lymphocyte subsets or in the CD4/8 ratios in the BALF samples from HTLV-1 carriers compared to control subjects.

HBZ and tax mRNA expression in BAL cells

To study the association between the *tax* and *HBZ* mRNA transcripts in HTLV-1 patients with pulmonary involvement, we performed qPCR assays. We examined 14 patients, seven with a DPB-like pattern and seven with the NSIP pattern in the CT findings. *HBZ* mRNA was detected in the BAL cells from 13 of the 14 patients (Fig. 2A), and *tax* mRNA was detected in 12 of the 14 patients (Fig. 2B); whereas there was no mRNA for either molecule detected in the BAL cells from control subjects. There were no significant differences

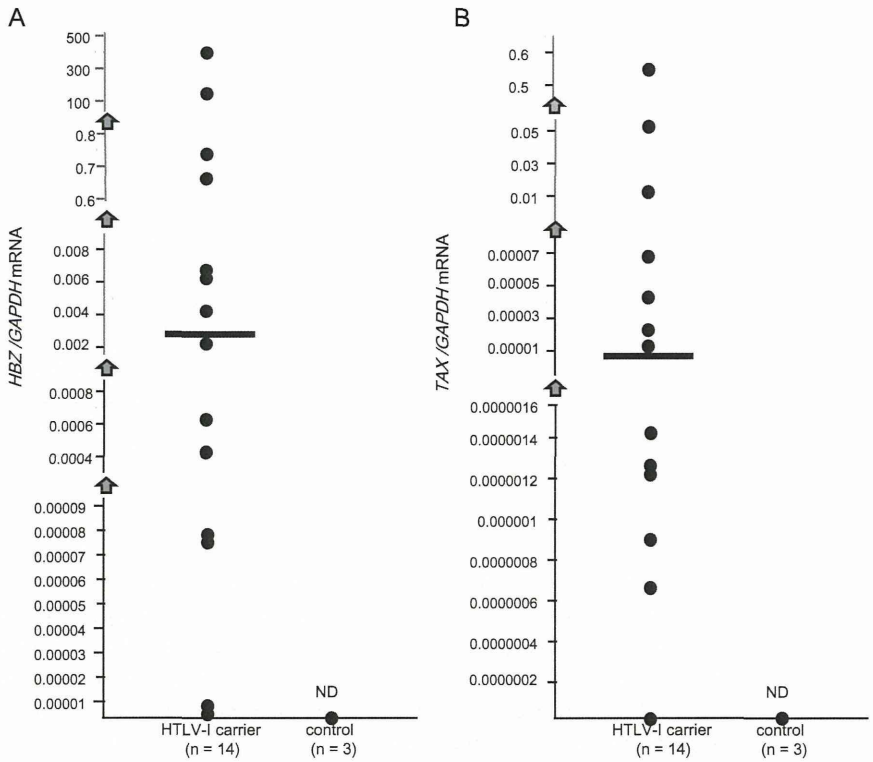

Figure 2. Detection of HTLV-I *tax* mRNA and *HBZ* mRNA in the bronchoalveolar lavage cells from patients with HTLV-I-associated lung disorders. (A) *HBZ* mRNA was detected in the BAL cells from 13/14 patients (median 0.00327, range 0–462.439). (B) Transcripts corresponding to *tax* mRNA were detected in the BAL cells from 12/14 patients (median 0.00000977, range 0–0.0568). There was no correlation between the *tax* and *HBZ* mRNA expression levels in the BAL cells from patients with HTLV-I-associated lung disorders.

of the expression levels of *HBZ* and *tax* mRNA in the BAL cells between patients with the DPB-like and NSIP patterns (data not shown). There was no significant correlation between the *tax* and *HBZ* mRNA expression levels.

Foxp3 mRNA in the BAL cells and lymphocytes in the BALF

We used qPCR assays to examine the association between the *Foxp3* mRNA transcripts and lymphocytes with the pulmonary involvement in HTLV-I-infected patients. We examined seven patients with a DPB-like pattern (cases 23, 25, 27, 30, 34, 35 and 40, Table 1), and four patients with the NSIP pattern (case 12, 14, 16 and 19, Table 1) in the CT findings and three control subjects (cases 40, 42 and 43, Table 1). The expression of *Foxp3* mRNA transcripts in the BAL cells was increased in the patients compared with that in the control subjects, although the difference was not statistically significant (Fig. 3A). The expression of *Foxp3* mRNA transcripts in the BAL cells positively correlated with the proportion of lymphocytes in the BALF, although the correlation was not statistically significant (Fig. 3B). The expression levels of *Foxp3* mRNA seemed to be higher in patients with the NSIP pattern compared with those with the DPB-like pattern (701.84 and 21.90 respectively; p=0.211), although there was no significant difference between the groups.

Cytokine concentrations in the BALF

There were significant increases in the expression levels of T helper cell type 1 (Th1) related cytokines, including interleukin (IL)-2, IL-12 and interferon (IFN)-γ, in the BALF from HTLV-I carriers with pulmonary involvement compared with control subjects (Fig. 4A-C). Additionally, the IL-10 levels were also increased in the BALF from HTLV-I carriers with pulmonary involvement compared with control subjects (Fig. 4D).

Discussion

In this study, we demonstrated that there was increased expression of *tax* and *HBZ* mRNA in the BAL cells from patients with HTLV-I-associated lung disorders. Additionally, we showed that a pattern of NSIP in chest CT scans could be one manifestation of HTLV-I-associated lung diseases. A positive correlation was observed between the expression of *Foxp3* mRNA and the percentages of lymphocytes in the BALF of patients with HTLV-I-associated lung disorders.