Elevated Levels of Soluble Adhesion Molecules in Sera and BAL Fluid of Individuals Infected With Human T-Cell Lymphotropic Virus Type 1*

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Study objective: T-lymphocytic alveolitis and increased levels of interleukin-2 receptor-α (CD25)-bearing T cells in the BAL fluid (BALF) of human T-cell lymphotropic virus type 1 (HTLV-1) carriers have been reported. Several chemokines and adhesion molecules may contribute to the accumulation of T lymphocytes in the lungs of HTLV-1 carriers. To clarify the correlation between adhesion molecules and HTLV-1-associated pulmonary disorders, we compared the distribution of T-lymphocyte subsets and soluble adhesion molecules, including soluble intercellular adhesion molecule (sICAM)-1, soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble L-selectin (sL-selectin), soluble E-selectin (sE-selectin), and soluble P-selectin (sP-selectin), in BALF and peripheral blood, between HTLV-1 carriers and noninfected healthy subjects.

Design: Flow cytometric analysis with monoclonal antibodies to cell-surface antigens was used to identify T-lymphocyte subsets in BALF samples from HTLV-1 carriers (n = 13) and noninfected healthy control subjects (n = 10). The levels of various soluble adhesion molecules in serum and in BALF were estimated by enzyme-linked immunosorbent assay.

Results: Higher percentages of CD3+ cells, CD3-expressing human leukocyte antigen-DR antigen, and CD3+CD25+ cells were detected in the BALF of HTLV-1 carriers than in that of noninfected control subjects. The concentrations of sICAM-1, sVCAM-1, sL-selectin, sE-selectin, and sP-selectin in the sera of patients were significantly higher than those in noninfected healthy control subjects. The concentration of sICAM-1 in the BALF of patients was significantly higher than that in noninfected healthy control subjects, and the concentration of sICAM-1 correlated well with the percentage of CD3+CD25+ cells.

Conclusion: The concentrations of adhesion molecules in the sera of and sICAM-1 in the BALF of HTLV-1 carriers were significantly higher than those in noninfected individuals, and the concentration of sICAM-1 correlated well with the percentage of CD3+CD25+ cells in BALF. Our results suggest a possible interaction between activated T cells bearing CD25 and soluble adhesion molecules, especially sICAM-1, which may contribute to the pulmonary involvement in HTLV-1 carriers.

Key words: adhesion molecules; BAL; human T-cell lymphotropic virus-1 carriers; interleukin-2 receptor-α

Abbreviations: AC = asymptomatic carrier; ATL = adult T-cell leukemia; BALF = BAL fluid; FITC = fluorescein isothiocyanate; HAM/TSP = human T-cell lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis; HAU = human T-cell lymphotropic virus type 1-associated uveitis; HLA = human leukocyte antigen; HTLV-1 = human T-cell lymphotropic virus type 1; ICAM = intercellular adhesion molecule; IL = interleukin; IL-2Ra = interleukin-2 receptor-α; MoAb = monoclonal antibody; PBS = phosphate-buffered saline solution; PE = phycoerythrin; sE-selectin = soluble E-selectin; sICAM = soluble intercellular adhesion molecule; sL-selectin = soluble L-selectin; sP-selectin = soluble P-selectin; sVCAM = soluble vascular cell adhesion molecule; VCAM = vascular cell adhesion molecule

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Human T-cell lymphotropic virus type 1 (HTLV-1) is the causative agent of adult T-cell leukemia (ATL). It is also associated with several nonmalignant disorders such as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), HTLV-1-associated uveitis (HAU), Sjögren’s syndrome, and arthrop-
athy with pulmonary involvement.\textsuperscript{1–5} With regard to pulmonary involvement in HTLV-1 infection, several studies from HTLV-1 pandemic areas have reported that patients with HAM/TSP and HAU develop frequent pulmonary complications during the carrier state characterized by nonmalignant T-lymphocytic alveolitis, and a similar pulmonary involvement has even been observed in asymptomatic carriers (ACs).\textsuperscript{2–6} Unlike that in patients with ATL, pulmonary involvement does not correlate with leukemic cell infiltration, and pathogens associated with opportunistic infections are not found in the lungs of these HTLV-1-infected individuals. Pulmonary complications in these individuals are characterized by T-lymphocytic alveolitis or lymphocytic interstitial pneumonia, which are associated with increased levels of interleukin (IL)-2 receptor-α (IL-2Rα; also called CD25\textsuperscript{+})-positive T cells and by marked elevation of soluble IL-2Rα levels in BAL fluid (BALF).\textsuperscript{7–9} The results of these studies have suggested that HTLV-1 infection could induce chronic inflammation in the lung through immunologic mechanisms.

HTLV-1-infected T cells are known to produce and release various types of cytokines, chemokines, and adhesion molecules.\textsuperscript{10–16} HTLV-1-infected cells in the lung may induce local T-cell expansion and chronic inflammation through the actions of these molecules. Advances for the last several years in molecular and cellular immunology have shown that several adhesion molecules participate in the recruitment of inflammatory cells to the site of inflammation. Intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 are members of the Ig superantigen family. ICAM-1 acts as a ligand for B2 leukocyte integrins, such as lymphocyte function-associated antigen-1 and Mac-1,\textsuperscript{17,18} while VCAM-1 binds the very late antigen-4, which is one of the B1 integrins.\textsuperscript{19} Integrin adhesion to ICAM-1 or VCAM-1 is thought to stabilize the adhesion of leukocytes to the endothelium.\textsuperscript{20} The selectin family of adhesion molecules and their respective ligands are important in the early transit adhesion-rolling phase.\textsuperscript{20} The selectin family consists of three distinct carbohydrate receptors expressed on leukocytes (L-selectin), endothelium (E-selectin), and platelets and endothelium (P-selectin).\textsuperscript{21} In this regard, several studies have identified the soluble forms of these adhesion molecules in the sera and BALF of healthy subjects and patients with inflammatory lung diseases.\textsuperscript{22–26}

In the present study, we determined the concentrations of the soluble forms of these adhesion molecules in the sera and BALF of HTLV-1-infected individuals and correlated these levels with the percentage of CD3+CD25+ cells in BALF. Our results suggest that adhesion molecules in HTLV-1 carriers may contribute to the accumulation of T lymphocytes in the lung, leading to inflammation of the lung.

**Materials and Methods**

**Subjects**

The subject group consisted of 13 consecutive HTLV-1-seropositive patients who visited our hospital and received BAL analysis between 1995 and 1998. Seropositivity was examined using the gelatin particle agglutination method (Serodia-HTLV-1; Fuji Rebio; Tokyo, Japan) and was confirmed by immunoblotting. Two of 13 seropositive patients were HAM/TSP patients (2 men), and the remaining 11 patients were designated as carriers (4 men and 7 women; age range, 32 to 75 years). Although some patients showed 1 to 3% atypical lymphocytes in peripheral blood, leukocyte counts were within normal limits. The carrier group consisted of two patients with diffuse panbronchiolitis, one with pulmonary fibrosis, and eight ACs. Five of eight ACs had normal findings on chest roentgenograms, but the other three showed small reticular shadows at lower lung fields.

We also included 10 healthy control subjects who were seronegative for HTLV-1 (7 men and 3 women; age range, 20 to 55 years). Informed consent was obtained from all patients and volunteers.

**BAL and Preparation**

BAL was performed using a standard technique. Briefly, the patient was first premedicated IM with atropine (0.5 mg). After local anesthesia with 4% lidocaine, a flexible fiberoptic bronchoscope (bronchofiber scope-P20; Olympus; Tokyo, Japan) was wedged into a subsegment of the right middle lobe for lavage. An aliquot of 50 mL sterile physiologic saline solution at body temperature was instilled through the bronchoscope, and the fluid was immediately retrieved by gentle suction using a sterile syringe. The procedure was repeated three times. The collected BALF was passed through two sheets of gauze and was centrifuged at 40g for 10 min at 4°C, and the supernatant was stored at -80°C until used. After washing twice with phosphate-buffered saline (PBS) solution, cells were suspended in 10% heat-inactivated fetal calf serum and were counted using a hemocytometer. An aliquot of 50 mL sterile physiologic saline solution at body temperature was instilled through the bronchoscope, and the fluid was immediately retrieved by gentle suction using a sterile syringe. The procedure was repeated three times. The collected BALF was passed through two sheets of gauze and was centrifuged at 40g for 10 min at 4°C, and the supernatant was stored at -80°C until used. After washing twice with phosphate-buffered saline (PBS) solution, cells were suspended in 10% heat-inactivated fetal calf serum and were counted using a hemocytometer. An aliquot then was adjusted to 2×10\textsuperscript{6} cells/mL, and a 0.2-mL sample of each cell suspension was spun down onto an glass slide at 160g for 2 min using a cytocentrifuge (Cytospin 2; Shandon Instruments; Pittsburgh, PA). The slides were later dried, fixed, and then stained using the May-Giemsa method. Two hundred cells were identified under a photomicroscope. The remaining cells were resuspended in PBS supplemented with 10% fetal calf serum and were incubated in plastic flasks for 90 min at 37°C in humidified 5% CO\textsubscript{2}/air for the depletion of alveolar macrophages. The cells then were centrifuged at 500g for 5 min at 4°C, the supernatant was discarded, and the cells were resuspended in PBS. Cells then were washed twice in PBS, were passed through a 100-μm nylon mesh filter, and finally were adjusted to a density of 1×10\textsuperscript{6} cells/mL. Viable cells constituted >90% of nonadherent cells, which were collected for flow cytometric analysis using trypan blue exclusion test.

**Monoclonal Antibody**

Fluorescein isothiocyanate (FITC)-conjugated anti-CD4, CD8, anti-human lymphocyte antigen (HLA)-DR, anti-IL-2Rα (CD25)
monoclonal antibody (MoAb), and phycoerythrin (PE)-conjugated anti-CD3, CD4, and CD8 MoAb all were used in the study (Becton Dickinson; Mountain View, CA). Mouse IgG1 conjugated with FITC or PE (Becton Dickinson) was used to determine the borderline between stained and unstained cells in flow cytometric analysis.

**Two-Color Direct Immunofluorescence Staining**

Whole-blood samples were withdrawn by venipuncture from HTLV-1-infected individuals and noninfected healthy volunteers and were mixed with ethylenediaminetetraacetic acid. A portion of this sample (100 μL) was placed into a 12 × 5-mm polystyrene tube (Falcon Plastics; Oxnard, CA), and then 5 μL of each MoAb was added. The tubes were incubated for 15 min at room temperature in darkness, and 2 mL lysing solution (FACS; Becton Dickinson) was added once to destroy RBCs. The cells were mixed vigorously, incubated for 10 min at room temperature, then washed once in cold PBS containing 0.1% sodium azide. The cells were finally resuspended in cold PBS containing 0.5% paraformaldehyde.

The density of cells in BALF was adjusted to 1 × 10⁶ cells/mL. A volume of 5 μL of each MoAb was placed into a polystyrene tube, and 100 μL of cell suspension (1 × 10⁶ cells) was added. Cells were incubated for 30 min on ice in the dark, were washed once in cold PBS containing 0.1% sodium azide, and then were resuspended in cold PBS containing 0.5% paraformaldehyde. Fixed cells were kept in darkness at 4°C until analysis.

**Two-Color Flow Cytometry**

Stained cells were analyzed on a flow cytometer equipped with an argon ion laser set at 488 nm (FACScan; Becton Dickinson), and a computer system (Consort 30; Becton Dickinson) was used for data acquisition and analysis. A minimum of 10,000 events was collected for each sample. A cell gate containing lymphocytes was established on the basis of forward and side-light scatter. While flow cytometry is not an ideal technique because measurements are based on assumptions, lymphocytes were confirmed to consist of >90% CD3⁺ cells using anti-CD3 antibodies. To determine the borderline between stained and unstained cells, cells also were stained with mouse IgG1-conjugated FITC or PE. The FITC and PE fluorochromes were excited at the same wavelength (488 nm) with the argon laser beam. The FITC and PE fluorescence was collected using, respectively, 530- and 573-nm bandpass interference filters and dichroic mirrors. Electronic compensation was used to prevent contamination by FITC fluorescence in the PE channel. The percentage of each subset was calculated based on the number of lymphocytes found in each quadrant.

**Measurement of Soluble Adhesion Molecules**

Serum concentrations of soluble ICAM (sICAM)-1, soluble VCAM (sVCAM)-1, soluble L-selectin (sL-selectin), soluble E-selectin (sE-selectin), and soluble P-selectin (sP-selectin) were measured using enzyme-linked immunosorbent assay kits (Quantikine; R&D Systems; Minneapolis, MN). Serum samples were diluted 1:20 for sE-selectin, sP-selectin, and sICAM-1, 1:50 for sVCAM-1, and 1:100 for sL-selectin assays before each assay.

Sufficient quantities of BALF were available from HTLV-1 carriers and noninfected healthy volunteers for the estimation of sICAM-1, sVCAM-1, sL-selectin, sE-selectin, and sP-selectin levels after the concentration of the supernatant (Centricon-30 [used to concentrate low-molecular-weight components with a cutoff molecular weight of 3,000 d]; Amicon; Beverly, MA). In this concentration procedure, the recovery of each soluble adhesion molecule was >90%, and the magnification of concentration was calculated by the ratio of protein consistency in unconcentrated BALF supernatants to that in concentrated BALF supernatants, which was measured using the direct current protein assay kit (Bio-Rad Laboratories; Hercules, CA). The original level of the soluble adhesion molecules was later corrected by this ratio. The level of soluble adhesion molecules was quantified using enzyme-linked immunosorbent assay kits (Quantikine; R & D Systems). Since BAL has a dilutional effect on the recovery of the soluble adhesion molecules, measurements are occasionally standardized to albumin or urea. There was a good correlation between nonstandardized and standardized values by albumin concentration in BALF (r = 0.673; p = 0.005). Thus, the level of the soluble adhesion molecules was expressed using the measured level rather than that relative to albumin concentration. The detection limits were the following: sICAM-1, 2.0 pg/mL; sVCAM-1, 4.0 pg/mL; sL-selectin, 0.75 pg/mL; sE-selectin, 1.2 pg/mL; and sP-selectin, 0.8 pg/mL. Cross-reactions with other cytokines were not observed.

**Statistical Analysis**

All values were expressed as median (range). The Mann-Whitney U test was used to compare differences between unpaired samples, while the Wilcoxon signed rank test was used to compare differences between paired samples. Where necessary, the results of the analysis were further corrected using the Bonferroni method. Spearman rank correlation was used to examine the relationship between various parameters. Statistical analysis was performed using a software package (StatView, version J 4.5; SAS Institute; Cary, NC). A p value < 0.05 was considered to be significant.

**Results**

**Lymphocyte Subsets in BALF and Concentrations of Soluble Adhesion Molecules in Sera and BALF in HTLV-1 Carriers**

Lymphocyte subsets in BALF and concentrations of soluble adhesion molecules in sera and BALF in each of the 13 HTLV-1 carriers are depicted in Table 1. An analysis of lymphocyte subsets in BALF showed increased percentages of lymphocytes, CD3⁺HLA-DR⁺ cells, and CD3⁺CD25⁺ cells. Furthermore, the level of each soluble adhesion molecule in sera was higher than that in BALF.

**Lymphocyte Subsets in Peripheral Blood of HTLV-1 Carriers and Healthy Volunteers**

The percentages of CD3⁺, CD4⁺, and CD8⁺ cells and the CD4/CD8 ratio in peripheral blood were similar in HTLV-1 carriers and noninfected healthy volunteers (data not shown). However, the percentages of CD3⁺HLA-DR⁺ cells and CD3⁺CD25⁺ cells in peripheral blood were significantly higher in HTLV-1 carriers (CD3⁺HLA-DR⁺ cells, 17.4% [range, 10.3 to 29.0%]; CD3⁺CD25⁺ cells, 10.1% [range, 3.9 to 13.3%]) than in noninfected healthy control subjects.
The percentages of CD3⁺, CD3⁺HLA-DR⁺, and CD3⁺CD25⁺ cells were significantly higher in HTLV-1 carriers than in noninfected healthy volunteers. Furthermore, in HTLV-1 carriers, the percentages of CD3⁺, CD3⁺HLA-DR⁺, and CD3⁺CD25⁺ cells were significantly higher in BALF (CD3⁺ cells, 90.7% [range, 69.4 to 98.5%]; CD3⁺HLA-DR⁺ cells, 53.1% [range, 9.7 to 87.9%]; CD3⁺CD25⁺ cells, 20.3% [range, 8.7 to 51.3%]) than in peripheral blood (CD3⁺ cells, 68.7% [range, 56.2 to 83.6%]; CD3⁺HLA-DR⁺ cells, 17.4% [range, 10.3 to 29.0%]; CD3⁺CD25⁺ cells, 10.1% [range, 3.9 to 13.3%]; p < 0.05). The percentages of CD4⁺ cells and CD8⁺ cells and the CD4/CD8 ratio were, however, similar in the BALF of HTLV-1 carriers and noninfected healthy volunteers.

Lymphocyte Subsets in BALF of HTLV-1 Carriers and Healthy Volunteers

The volume of BALF for HTLV-1 carriers and noninfected healthy volunteers was the same (data not shown). A significantly higher number of total cells, percentage of lymphocytes, and absolute number of lymphocytes were present in the BALF of HTLV-1 carriers, as well as significantly lower percentage of alveolar macrophages, relative to the respective variables in healthy control subjects (data not shown). The absolute number of alveolar macrophages in HTLV-1 carriers was not different from that in noninfected healthy volunteers (data not shown). In addition, the percentages of neutrophils, eosinophils, and basophils in HTLV-1 carriers (2.2%, 1.4%, and 0.2%, respectively) were not different from those in noninfected control subjects (2.8%, 1.0%, and 0%, respectively).

The percentages of CD3⁺, CD3⁺HLA-DR⁺, and CD3⁺CD25⁺ cells were significantly higher in HTLV-1 carriers than in noninfected healthy volunteers. Furthermore, in HTLV-1 carriers, the percentages of CD3⁺, CD3⁺HLA-DR⁺, and CD3⁺CD25⁺ cells were significantly higher in BALF (CD3⁺ cells, 90.7% [range, 69.4 to 98.5%]; CD3⁺HLA-DR⁺ cells, 53.1% [range, 9.7 to 87.9%]; CD3⁺CD25⁺ cells, 20.3% [range, 8.7 to 51.3%]) than in peripheral blood (CD3⁺ cells, 68.7% [range, 56.2 to 83.6%]; CD3⁺HLA-DR⁺ cells, 17.4% [range, 10.3 to 29.0%]; CD3⁺CD25⁺ cells, 10.1% [range, 3.9 to 13.3%]; p < 0.05). The percentages of CD4⁺ cells and CD8⁺ cells and the CD4/CD8 ratio were, however, similar in the BALF of HTLV-1 carriers and noninfected healthy volunteers.

Concentrations of Serum sICAM-1, sVCAM-1, sL-Selectin, sE-Selectin, and sP-Selectin

Table 2 shows the mean concentrations of sICAM-1 and sVCAM-1 in serum samples obtained from HTLV-1 carriers and noninfected healthy volunteers. The concentrations of both ICAM-1 and VCAM-1 were significantly higher in HTLV-1 carriers than in noninfected healthy control subjects. Moreover, the concentrations of sL-selectin, sE-
selectin, and sP-selectin were also significantly higher in HTLV-1 carriers than in noninfected healthy control subjects.

**BALF Concentrations of sICAM-1, sVCAM-1, sL-Selectin, SE-Selectin, and sP-Selectin**

sVCAM-1 was detectable only in the BALF of HTLV-1 carriers but not in that of healthy subjects, although sICAM-1 was detectable in the BALF of both HTLV-1 carriers and healthy subjects. sL-selectin, sE-selectin, and sP-selectin were not detectable in the BALF of all subjects, although they were detectable more frequently in HTLV-1 carriers (sL-selectin, 10 patients; sE-selectin, 4 patients; and sP-selectin, 2 patients) than in healthy subjects (sL-selectin, 6 patients; sE-selectin, 0 patients; and sP-selectin, 0 patients). The concentration of sICAM-1 was significantly higher in HTLV-1 carriers than in noninfected healthy control subjects (Fig 1, left, A). There was no significant correlation between the concentrations of the same soluble adhesion molecules in serum and in BALF (data not shown). BALF concentrations of soluble adhesion molecules were not reflected by serum concentrations.

**Correlation Between Concentrations of Soluble Adhesion Molecules and CD3+CD25+ Cells in BALF**

Based on the above results, we next examined the relationship between concentrations of soluble adhesion molecules and percentages of CD3+ lymphocytes or CD3+CD25+ cells in the BALF of HTLV-1 carriers. Unfortunately, we could not examine the relationship between the concentrations of sL-selectin, sE-selectin, and sP-selectin and the percentage of lymphocyte subsets in BALF, because the concentrations of these adhesion molecules were not detectable in the BALF of all HTLV-1 carriers and healthy volunteers. There was no significant correlation between percentage of CD3+ lymphocytes and concentrations of sICAM-1 or sVCAM-1 (data not shown). There was also no significant correlation between the absolute number of lymphocytes and the concentrations of soluble adhesion molecules (data not shown). However, there was a significant correlation between the percentage of CD3+CD25+ cells and the concentration of sICAM-1, but not that of sVCAM-1, in the BALF of HTLV-1-infected individuals (r = 0.661; p < 0.05; Fig 1, right, B).

**Discussion**

HTLV-1 infection is associated not only with ATL, but also with a variety of nonneoplastic inflammatory diseases, such as HAM/TSP and HAU. Patients with these HTLV-1-associated diseases are also known to develop pulmonary complications.2–5 The latter are also frequently observed in asymptomatic healthy HTLV-1 carriers.2–6 Accordingly, a new clinical entity, termed HTLV-1-associated bronchopneumopathy/bronchioalveolitis, has been suggested.2,6

**Figure 1.** The concentration of sICAM-1 in the BALF of healthy (normal) subjects and HTLV-1 carriers. Left, A: the concentration of sICAM-1 in the BALF of HTLV-1 carriers was significantly higher than in that of healthy subjects. The whisker box plots represent the 25th to 75th percentile of results inside the box, the median is indicated by the horizontal bar across the box, and whiskers on each box represent the 10th to 90th percentiles. **p < 0.01; *p < 0.05 (Mann-Whitney U test). Differences were still significant after correction for multiple testing using the Bonferroni method. Right, B: correlation between the percentage of CD3+CD25+ cells and the concentration of sICAM-1 in the BALF of HTLV-1 carriers. The concentration of sICAM-1 significantly correlated with the percentage of CD3+CD25+ cells in the BALF of HTLV-1-infected individuals (r = 0.661; p < 0.05).
Several studies have postulated that immunologic mechanisms may play an important role in the pathogenesis of these HTLV-1-associated diseases, since HTLV-1 tax, which is encoded by the pX region of the HTLV-1 proviral genome, could induce the expression of host cellular genes including IL-2, IL-2Rα (CD25), and several other cytokines and chemokines, as well as the HTLV-1 gene. In fact, the levels of soluble IL-2Rα, tumor necrosis factor-α, and interferon-γ are elevated in the cerebrospinal fluid of patients with HAM/TSP, suggesting a role for these cytokines in the inflammation of spinal cord lesions. As for pulmonary lesions, our group has previously demonstrated the presence of high percentages of lymphocytes, CD3+CD25+ cells, and HLA-DR+ cells (a T-cell activation marker) in the BALF of HTLV-1 carriers relative to those in healthy subjects. These results indicated an accumulation of T lymphocytes in the lung and confirmed the presence of inflammation of the lung in HTLV-1 carriers. Moreover, we demonstrated also the presence of high levels of β-chemokines in the BALF of HTLV-1-infected individuals relative to healthy noncarriers and that the concentration of β-chemokines in BALF and the level of HTLV-1 tax messenger RNA expression in BAL cells significantly correlated with the percentage of CD3+CD25+ cells in BALF.

Several studies reported that HTLV-1-infected cells produced not only cytokines but also adhesion molecules. The overexpression of genes of adhesion molecules and transcriptional transactivation of its promoter by HTLV-1 tax were reported in cultured HTLV-1-infected cells. The involvement of adhesion molecules also has been suggested in the progression of HTLV-1-associated disorders. For example, increased concentrations of soluble adhesion molecules in the cerebrospinal fluid of HAM/TSP patients correlated with disease progression.

In the present study, we measured the concentrations of soluble adhesion molecules in the serum and BALF of HTLV-1-infected individuals and noninfected healthy subjects. Our results showed that the concentrations of soluble adhesion molecules in serum were significantly elevated in HTLV-1-infected individuals compared to noninfected healthy subjects. These data suggested that soluble adhesion molecules may be directly involved in the pathogenesis of HTLV-1-associated disorders in HTLV-1-infected individuals. Our results also showed that the concentration of sICAM-1 was significantly higher in the BALF of HTLV-1 carriers than in that of noninfected healthy control subjects and that sVCAM-1 was detectable only in the BALF of HTLV-1 carriers. These data suggested that both ICAM-1 and VCAM-1 may be directly involved in the pathogenesis of T-cell alveolitis in the lungs of HTLV-1-infected individuals. In particular, since there was significant correlation between the percentage of CD3+CD25+ cells and the concentration of sICAM-1 in BALF, sICAM-1 may be involved in the pathogenesis of T-cell alveolitis in the lungs of HTLV-1-infected individuals, although the increased sICAM-1 levels could also just reflect the increased movement of cells into the lung.

ICAM-1 is expressed on the surface of various cells, including vascular endothelial cells, epithelial cells, and lymphocytes. The ICAM-1 that sheds from cell surfaces can be detected as a soluble form. The ICAM-1-lymphocyte function-associated antigen-1 interaction is important in T-lymphocyte activation and in lymphocyte migration into the site of inflammation. Previous studies have shown elevated sICAM-1 levels in serum and BALF samples from patients with various interstitial lung diseases, and that measurement of sICAM-1 is useful for assessing and monitoring lung disease activity in these patients. The expression of ICAM-1 on the cell surface is induced by HTLV-1 trans-activated proinflammatory cytokines, such as tumor necrosis factor-α and IL-1β. The shedding of ICAM-1 from the cell surface is also enhanced by these cytokines.

In the present study, we showed significantly high concentrations of sL-selectin, sE-selectin, and sP-selectin in the sera of HTLV-1 carriers relative to those in healthy subjects, and the concentrations were detectable more frequently in HTLV-1 carriers than in healthy subjects. These results suggest that selectins are involved in the pathogenesis of HTLV-1-associated disorders. Selectins are involved in the capture of cells at inflammatory sites by initiating their rolling on the endothelium. L-selectin is expressed on most leukocytes and sL-selectin levels were up-regulated in patients with various lung diseases when compared to healthy subjects. These studies suggest that L-selectin levels are related to chronic inflammatory diseases in the lung. In this regard, Pizcueta and Luscinskas reported that the anti-L-selectin MoAb inhibited lymphocyte accumulation in patients with acute as well chronic inflammation. Furthermore, inositol polyanions, simple 6-carbon ring noncarbohydrate small molecules derived from D-myo-inositol (1,2,3,5-trans-4,6, cyclohexanehexol), inhibit L-selectin and P-selectin function in vitro and inflammation in vivo. These studies suggest that anti-adhesion molecule therapy may have some beneficial effects against pulmonary involvement in HTLV-1 carriers.

In conclusion, we demonstrated the presence of high percentages of activated T cells bearing CD25 and high concentrations of soluble adhesion mole-
cules in the lungs of HTLV-1 carriers as well as high concentrations of soluble adhesion molecules in the sera of HTLV-1 carriers. Our results suggest that the interaction between these inflammatory cells and adhesion molecules may contribute to the pulmonary involvement in HTLV-1 carriers. Since we could not define a direct effect for adhesion molecules on T cells in the BALF of these HTLV-1 carriers, further studies are required to define the role of memory T cells and adhesion molecules.

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