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

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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 diseases. 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.

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
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-1β 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 leukemic 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 1×10⁶ 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 (10⁵ cells per well) were deposited on MT-2 supernant-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; Hishima 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%
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 preincubated 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.

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Triton X-100. The cells were washed with PBS/7% FBS and resuspended in PBS/7% FBS containing mouse mAb against Tax (1-t; 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 DM6000 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 B-7) (Santa Cruz Biotecnology), 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 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-κ B element of the IL-2 receptor β- or AP-1-binding site, and a typical B element of the IL-8 gene (5′-gatcGTGA-3′) gene (5′-tcgaTAGCTT-3′) and an activator protein-1 (AP-1) element of the IL-8 gene (5′-gatcGTGA ACTCAAGGT-3′). Underlined sequences represent the NF-κ B- or AP-1-binding site, and lower-case letters indicate residues added for labelling purposes.

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 32P-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′-tcgaTAGCTGAAATCTCCGGAGC-3′), a typical NF-κB element from the IL-2 receptor z-chain (IL-2Rz) gene (5′- gatcGGGAGGGGACTCCTCTCCTG-3′) and an activator protein-1 (AP-1) element of the IL-8 gene (5′-gatcGTGA ACTCAAGGT-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.
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). 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 (i) and C5/MJ (ii) supernatant (sup). 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.
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 pretreated 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
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).

To determine the precise regions of the ICAM-1 promoter necessary for Tax-mediated activation, an NF-κB site mutant (pGL1.3κB2) was co-transfected. Mutation of the NF-κB site abolished the Tax-mediated activation of the ICAM-1 promoter (Fig. 6d). 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. These results suggested that Tax transactivates the ICAM-1 gene via the NF-κB-binding site.

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 ζ-chain in human T-cell lines. Cells were reacted with FITC-labelled anti-LFA-1 ζ-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.
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
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-kB-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-kB-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.
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...
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 disorders 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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