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Short communication

Progressive multifocal leukoencephalopathy and CD4+ T-lymphocytopenia in a patient with Sjögren syndrome

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Abstract

We report progressive multifocal leukoencephalopathy (PML) and CD4+ T-lymphocytopenia in a 71-year-old man with Sjögren syndrome (SjS). The patient was admitted to our hospital because of progressive dementia and gait disturbance. T2-weighted MR images showed high-intensity lesions in his left frontal white matter thalamus, cerebellum and brainstem. A pathological diagnosis of PML was made by brain biopsy. SjS is frequently accompanied with immunological complications; however, there are few reports on PML in patients with SjS. Recently, isolated CD4+ T-lymphocytopenia is reported to be one of the based immunological conditions associated with the development of PML. In the present case, CD4+ T-lymphocytopenia was also observed on admission, which is also associated with SjS.

Keywords: JC virus; Progressive multifocal leukoencephalopathy; Sjögren syndrome; CD4+ T-lymphocytopenia; ^{123}I-IMP-SPECT; Brain biopsy

1. Introduction

Progressive multifocal leukoencephalopathy (PML), which is caused by the JC virus (JCV), usually develops as a central nervous system (CNS) opportunistic infectious disease in immunocompromised patients, such as those with acquired immunodeficiency syndrome (AIDS), a variety of collagen diseases, or those who are undergoing steroid hormone or immunosuppressive therapy [1]. Sjögren syndrome (SjS) is one of the collagen diseases and highly accompanied with immunological complications. However, to date, the disease has not been considered to be a high risk factor of PML. Here we report a rare case of PML developing in an aged patient with SjS accompanied with CD4+ T-lymphocytopenia.

2. Case report

The patient, a 71-year-old Japanese man with a 5-year history of primary SjS, was admitted to our hospital because of progressive dementia and gait disturbance that were observed 2 months and 1 month before his visit, respectively. His first symptom of SjS was dry mouth. His diagnosis of SjS was made 5 years ago by Saxon and Shirmer tests, scintigraphy of the parotid and submandibular glands, and lip biopsy that revealed many lymphocytes and plasma cells infiltrating the small salivary glands. Several months after the diagnosis of SjS, he developed interstitial pneumonia associated with SjS and required oxygen therapy at home. Since he was suffering from myocardial infarction, and

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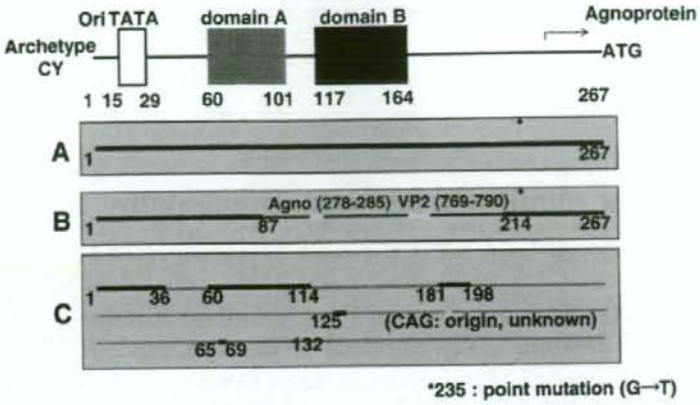

Fig. 1. The nested PCR for transcriptional control region (TCR) of the JCV test detected three types of TCR in the CSF. A: archetype with point mutation (235 G→T) virus. B and C: two different types of rearrangement of TCR virus.

chronic ischemic heart disease, he was not administrated corticosteroid or immunosuppressive therapy for SjS at any time before he visited our hospital.

On admission, he was afebrile, his blood pressure was 130/64 mmHg, his heart rate was 70 bpm and regular, his heart sounds had systolic murmur (Levine 3/IV), his respiratory sounds had fine crackles in the bilateral inferior areas of the chest, and no cervical or axially lymph nodes were palpable. Neurological examination revealed dementia, right hemiparesis, truncal and limb ataxia, normal deep tendon reflex, positive Babinski and Chaddock signs on the right side, and forced grasping in both hands.

Laboratory tests of his peripheral blood showed normal WBC counts with low percentage of lymphocytes; his CD4+ T-lymphocyte count of 272 cells/μl (%CD4; 16.1% (normal, 25–60%)) with a decline to 217 cells/μl (%CD4; 13.6%) within 1 month; and C-reactive protein level was 2.68 mg/dl. The serological tests for antibodies against HIV-1/-2, HTLV-1 and HBs were negative. The serological tests for anti-HCV antibodies were weakly positive; the result of the HCV RNA–PCR was negative. The syphilis serological tests (TPHA, STS) were negative. Autoimmune serological examination demonstrated 1280× antinuclear antibodies. However, the serological tests for other antibodies, including those to Rheumatoid factor, ds-DNA-IgG, Sm, SS-A, SS-B, RNP, and Scl-70, were entirely negative. Other laboratory findings were unremarkable, including the tumor markers. Analysis of his CSF revealed normal pressure and normal parameters with respect to cell count and chemical analysis (cell count; 3 mononuclear cell /μl, protein concentration; 24 mg/dl, glucose concentration; 56 mg/dl). Concentration of soluble IL2-receptor, β2 microglobulin and ferritin were not elevated in CSF. Oligoclonal bands (OCBs) were detected in CSF. Nested PCR revealed 3 types of JCV transcriptional genomes, including an archetype in CSF (Fig. 1). Whole body CT and ^{67}Ga-citrate scintigraphy revealed no malignancy in his body.

Brain MRI showed T1-weighted images with low-intensity lesions, and T2-weighted and FLAIR images with high-intensity lesions in the left frontal white matter, bilateral parietal and left occipital white matter, left thalamus, and right middle cerebellar peduncle, which were not enhanced by Gadolinium (Fig. 2). Spinal cord MRI showed no obvious abnormalities. Serial images of early, 6-hour delayed and 24-hour delayed ^{123}I-IMP SPECT (IMP-SPECT) showed a gradual increase in radioactivity and a gradual increase in amount and a long-term retention of the tracer in the left frontal white matter (Fig. 3). Brain biopsy from the left frontal deep white matter revealed disseminated enlarged oligodendrocytic nuclei that were immunopositive with rabbit polyclonal antibody against JCV-VP1 protein (Fig. 4) [2], JCV-agnoprotein and a large T protein (data not shown).

Treatments with steroid pulse therapy (methylprednisolone 1 g/day, 3 days) and cytarabine (AraC 2 mg/kg/day, 5 days) were not effective, and the patient developed akinetic mutism approximately 3 months after the onset of PML, and

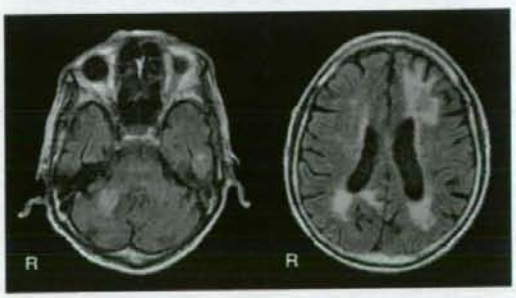

Fig. 2. Axial brain MRI on 1.5-tesla demonstrating multifocal high-intensity areas in left frontal and bilateral periventricular white matters, and right middle cerebellar peduncle on FLAIR (TE 144, TR 8002, TI 2000) images.

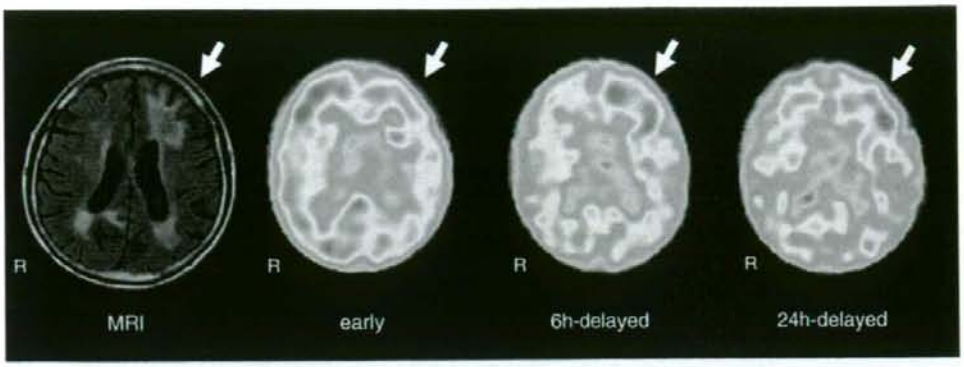

Fig. 3. Axial FLAIR MRI and Serial images of ^{123}I-IMP SPECT: early image, 6-hour delayed image and 24-hour delayed image. These serial images shows a delayed increase of radioactivity, a gradual increase and long term retention of the tracer on the left frontal white matter.

he has been alive for 18 months without any improvement in clinical state.

3. Discussion

We have described a case of PML with SjS, in which progressive dementia, right hemiparesis, and truncal and limb ataxia were the clinical features.

In this case, tumefactive multiple sclerosis (MS), cerebral vasculitis with SjS and primary CNS malignant lymphoma were initially considered as differential diagnoses from the MRI findings.

IMP-SPECT images demonstrated delayed high accumulation of IMP in the left frontal white matter. Primary CNS malignant lymphoma, malignant astrocytoma, and metastatic brain tumors, including malignant melanoma, have been reported to be the lesions to show high accumulation of IMP on delayed IMP-SPECT images [3–6]. Compared with that in normal brain tissues, a small amount of IMP is retained in tumor cells due to the differences in the number of amine receptors, fat distribution and tissue pH [6]. Recently it has been reported that IMP-SPECT shows the delayed accumulation of IMP in tumefactive MS lesions. Therefore, it appears to be a pitfall for distinguishing between MS and malignant lymphoma [7]. The mechanism underlying IMP delayed accumulation is still unclear, but we speculate a small amount of IMP is retained in JCV infected oligodendrocytes as well as in tumor cells.

OCBs were detected in our patient's CSF. OCBs are frequently detected in MS, and in some infectious diseases (meningitis, subacute sclerosing panencephalitis (SSPE), and PML), and other neurological diseases. OCBs observed in SSPE and PML are measles virus-specific [8] and JCV-specific IgG antibodies [9], respectively. Detecting OCBs in CSF is associated with intrathecal synthesis of IgG, and is not pivotal in distinguish between MS and other neurological diseases.

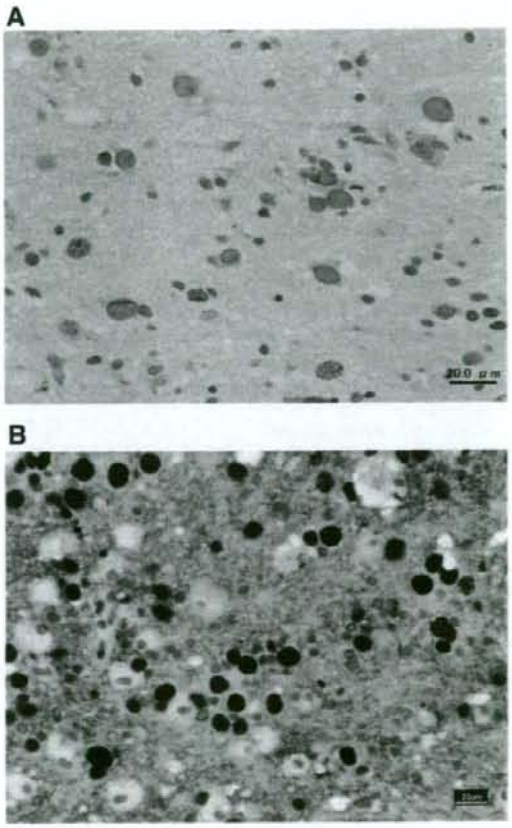

Fig. 4. (A) Brain section stained with hematoxylin-eosin, showing enlarged oligodendrocytic nuclei filled with somewhat basophilic substance in the white matter. Bar 20 μm. (B) Brain section immunostained with antibody against JCV-VP1, demonstrating that such enlarged oligodendrocytic nuclei are clearly positive for this protein. Bar 20 μm.

In the present case, we eventually considered that brain biopsy was necessary for making an accurate diagnosis of the brain lesions; a small brain tissue was collected from the left frontal white matter, where delayed accumulation of IMP was evident. The biopsy specimen showed many enlarged oligodendrocytic nuclei immunopositive for JCV-VP1 protein in demyelinated lesions. Subsequently, the presence of PML types of JCV DNA was also detected in the patient's CSF. Interestingly, the other Sjögren syndrome's patient with PML was from Japan [10]. This case was associated with acute myelocytic leukemia during the clinical course of SjS, and SjS itself was not considered to be the basic disease [10].

Recently, several reports have shown the development of PML in patients with idiopathic CD4+ T-lymphocytopenia (ICL) [11–16]. ICL is a recently described syndrome characterized by a marked decrease in the number of circulating CD4+ T-lymphocytes in the absence of any identifiable causes of immunologic abnormalities, and can be defined by the presence of a documented absolute number of CD4+ T-lymphocytes $<300/\mu l$ or a CD4+ cell count of <20% of the total T cells on two occasions, no evidence of HIV infection, and the absence of any defined immunodeficiency or therapy leading to the decrease in the CD4+ T cell level [17]. It has also been reported that according to the definition mentioned above, 5.2% of SjS patients have CD4+ T-lymphocytopenia [18]. Therefore, we also studied the presence or absence of this condition in the present case. As a result, the laboratory data obtained fulfilled the definition of this syndrome.

The patient has been alive but has not shown any improvement for 18 months since the onset of the disease: this is an unusually long course for the disease. Generally, the clinical course of PML in the absence of an HIV infection is rapidly progressive, and most patients die within a year. On the other hand, it was reported that PML patients with ICL tend to survive for over a year [12,14,15].

In conclusion, it is now necessary to consider PML as one of the differential diagnoses in patients with SjS showing leukoencephalopathy and also to study the number of CD4+ T cells in such patients.

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REGULAR ARTICLE

Inhaled corticosteroid therapy reduces cytokine levels in sputum from very preterm infants with chronic lung disease

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Keywords
Chronic lung disease, Cytokines, High-frequency oscillatory ventilation, Inhaled corticosteroids, Sputum

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Abstract

Aim: To evaluate the effects of inhaled corticosteroid therapy and high-frequency oscillatory ventilation (oscillation) on preterm infants with chronic lung disease (CLD).

Methods: Ten infants with CLD who received inhaled corticosteroid therapy were enrolled. Week 1 was defined as the first week of therapy. The concentrations of interleukin (IL)-8, tumour necrosis factor-α (TNF-α), IL-1β, IL-6, IL-10 and IL-12p70 in serial sputum specimens from the infants were determined using a cytometric bead array.

Results: The sputum concentrations of IL-8 obtained from the infants during week 3–4 were significantly lower than those obtained before therapy and during week 1–2. The sputum concentrations of TNF-α, IL-6 and IL-10 during week 3–4 were significantly lower than the concetrations during week 1–2. The ratio of IL-8 levels during week 1–2 to those before therapy in infants who received oscillation (n = 4) was significantly lower than in those who received intermittent mandatory ventilation (n = 6).

Conclusion: Inhaled corticosteroids may be associated with a decrease in pro-inflammatory cytokine levels in sputum from infants with CLD from 2 weeks after the start of therapy. Our further investigations suggest that therapy with oscillation modulated airway inflammation earlier than therapy with intermittent mandatory ventilation.

INTRODUCTION

Chronic lung disease (CLD) is an important problem in preterm infants who require assisted ventilation. It was initially reported in 1967 as bronchopulmonary dysplasia following ventilator therapy of respiratory distress syndrome (RDS). (1) Mechanical ventilation can injure the preterm lungs. Barotrauma, volutrauma and oxygen toxicity during intermittent positive-pressure ventilation are thought to be important factors in the pathogenesis of CLD. In general, most preterm infants are ventilated with intermittent mandatory (mandate) or high-frequency oscillatory (oscillation) mode. Although inhaled corticosteroid therapy is used in order to try to treat and prevent CLD in preterm infants, the mechanisms of any effects are still unclear.

Cytokines play an important role in the inflammatory response to airway injury, and the subsequent development of CLD, in preterm infants. (2) Multiple pro-inflammatory and chemotactic factors are present in the air spaces of ventilated preterm infants who develop CLD, and these factors are thought to be involved in the pathogenesis of CLD. (3,4)

To evaluate the anti-inflammatory effects of inhaled corticosteroid therapy on airway inflammation of infants during mechanical ventilation, we determined the levels of tumour necrosis factor-α (TNF-α), interleukin (IL)-1β, Il-6 and IL-8, which are proinflammatory cytokines, (5–7) IL-10, an anti-inflammatory cytokine, (5,8) and IL-12p70, a cytokine required for the induction of Th1 immune responses, (9) in sputum samples from these patients. Moreover, the differences in sputum cytokine levels from infants receiving mandate or oscillation ventilation were also analysed.

METHODS

Subjects

Ten infants (four boys and six girls; mean gestational age, 25.7 weeks; mean birth weight, 745 g) with CLD who were admitted to our neonatal intensive care unit between April 2004 and March 2007 were enrolled in this study (Table S1). Parental informed consent was obtained for all infants. The protocol was approved by the Institutional Review Board of Yamaguchi University Hospital (H16–134). All infants were intubated and mechanically ventilated immediately after birth because of RDS, which was diagnosed by typical chest radiograph and clinical signs of respiratory distress and received surfactant therapy within 1 h. All infants received neonatal care in accordance with standard practice for CLD in our neonatal intensive care unit (i.e. maintenance fluid dose, and use of theophylline and diuretics). CLD was defined as a requirement for supplemental oxygen or mechanical ventilation at 28 days of age, or beyond 36 weeks post conception age, to maintain PaO_2 > 50 mmHg. Inhaled corticosteroid therapy (fluticasone propionate 50 μg) twice a day was started from 8 days to 39 days after birth to extubation.

Collection of sputum samples

All samples of sputum were collected during routine endotracheal suction, which was being performed as part of normal medical care. Suction catheters were inserted just beyond the distal tip of the endotracheal tube. Suction was applied and the aspirated material was collected in a trap without any solution. The heart rate and respiratory rate were monitored and allowed to stabilize during the suctioning procedure. The first week after the start of inhaled corticosteroid therapy was considered as week 1. The treatment period was divided into three phases: 0–3 weeks before the therapy; during weeks 1–2; and during weeks 3–4. Sputum samples were obtained 2–4, 2–7 and 1–5 times during the three phases, respectively. The mean cytokine level for each phase was used. The samples were stored frozen at -30°C until analysis.

Determination of cytokine levels in sputum

The concentrations of IL-8, TNF-α, IL-1β, IL-6, IL-10 and IL-12p70 in the sputum samples were measured using a commercially available cytometric bead array kit (BD Pharmingen, San Diego, CA, USA) according to the manufacturer's instructions and as described previously. (10,11) Data analysis was performed using GraphPad Prism software (GraphPad Prism Software, San Diego, CA, USA). The lower detection limits for IL-8, TNF-α, IL-1β, IL-6, IL-10 and IL-12p70 were 3.6, 2.8, 7.2, 2.5, 2.8 and 1.9 pg/mL, respectively.

Statistical analysis

All data were log-transformed to obtain an approximately normal distribution. Differences in the results between groups were analysed by *t*-test, and those with p-values < 0.05 were considered significant. Correlations were analysed using Pearson's correlation coefficient. All values are shown as the geometric mean (GM), and all calculations were performed using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Characteristics of infants with CLD

All infants were diagnosed as having patent ductus arteriosus (PDA) by echocardiogram at birth. In patients 6, 8, 9 and 10, the PDA closed spontaneously within 2 days after birth and in patients 1, 3, 4 and 5, the PDA closed after one or two doses of indomethacin therapy. In patient 2, three doses of indomethacin were given. Patient 7 received prostaglandin therapy opening PDA for severe pulmonary stenosis. None of the infants had haemodynamically significant PDA or received ibuprofen and ligation. All infants were treated with theophylline and diuretics, from 2–77 (duration, 11–189 days) and 1–26 (duration, 60–192 days) days after birth, respectively. The infants were divided into two groups at the initiation of inhaled corticosteroid therapy: those ventilated with oscillation (n = 4); and those ventilated with mandate (n = 6) (Table S1). There were no significant differences in gestational age or birth weight between infants in the two ventilation groups. The inhaled corticosteroid therapy was administered to the infants for 16–91 days, with no significant side effects. Only two infants (patients 2 and 7) required home oxygen therapy, because of congenital heart disease (but not CLD). The other infants required supplemental oxygen for 3–42 days after extubation.

Cytokine levels in sputum

Cytokine levels in sputum from the infants before the inhaled corticosteroid therapy and during week 1–2 and week 3–4 after the start of the therapy are shown in Figure S1. The concentrations of IL-8 during week 3–4 (GM 417 pg/mL) were significantly lower than those before the inhaled corticosteroid therapy (GM 10,482 pg/mL; $p = 0.005$) and during week 1–2 (GM 8,894 pg/mL; $p = 0.005$; Fig. S1). The concentrations of TNF-α (GM 5.6 pg/mL), IL-6 (GM 29.2 pg/mL) and IL-10 (GM 3.7 pg/mL) during week 3–4 were significantly lower than those during week 1–2 (TNF-α, GM 37.8 pg/ml, $p = 0.041$; IL-6, 266.3 pg/mL, $p = 0.029$; IL-10, 17.2 pg/mL, $p = 0.038$). There were no significant correlations between the level of each cytokine before the inhaled corticosteroid therapy and the length of time taken for PDA closure or the start time of administration of theophylline or diuretics.

There was a correlation between TNF-α and IL-10 levels before the inhaled corticosteroid therapy ($p = 0.011$). There was no correlation for any of the cytokines during weeks 1–2 and 3–4.

Except for patients 2 and 7, who exhibited congenital heart disease, sputum IL-8 levels during week 3–4 correlated significantly with the supplemental oxygen period after extubation ($p = 0.041$; Fig. S2).

High-frequency oscillatory ventilation versus intermittent mandatory ventilation

The ratios of IL-8 levels during week 1–2/before the inhaled corticosteroid therapy in infants with oscillation were significantly lower than the ratios in those with mandate (GM 0.36 vs. 1.51 pg/mL; $p = 0.027$; Fig. 1). The ratios of IL-12p70 levels during week 1–2/before the therapy in infants with oscillation were significantly higher than the ratios in those with mandate (GM 2.60 vs. 0.01 pg/mL; $p = 0.010$; Fig. 1). The ratios of TNF-α, IL-1β, IL-6 and IL-10 during week 1–2/before the therapy, during week 3–4/before the therapy or during week 3–4/week 1–2 were not significantly different between infants who received the two ventilation modes.

DISCUSSION

The results of our present study demonstrate that levels of IL-8, TNF-α, IL-6 (pro-inflammatory cytokines) and IL-10 (an anti-inflammatory cytokine) were high in sputum from infants with CLD; however, we could not compare the values with normal controls. It is very difficult to obtain sputum from age-matched controls without CLD or infection. We have previously determined cytokine levels in sputum from healthy adult controls. (11) IL-8, TNF-α, IL-6 and IL-10

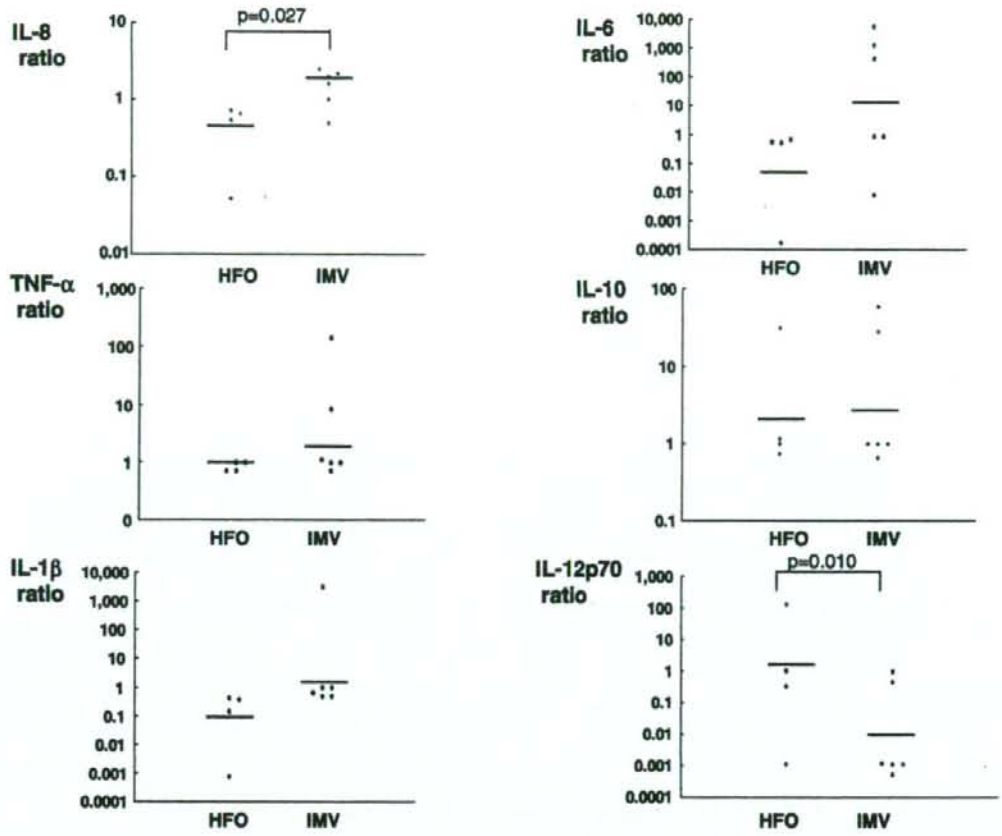

Figure 1 The ratios of IL-8, TNF-α, IL-1β, IL-6, IL-10 and IL-12p70 levels in sputum during week 1–2 to those before inhaled corticosteroid therapy in infants with CLD who received high-frequency oscillation (HFO) and intermittent mandatory ventilation (IMV). Horizontal bars indicate the geometric mean values.

levels in sputum from CLD infants before inhaled corticosteroid therapy were significantly higher than those in the healthy adults in our previous study. In addition, there were no significant differences in IL-8, TNF-α, IL-1β, IL-6, IL-10 or IL-12p70 levels in sputum in the infants at week 3–4 and in the healthy adult controls. Considering the results of our previous study, we suggest that these sputum cytokine levels from CLD infants before inhaled corticosteroid therapy were high. IL-8, TNF-α, IL-1β, IL-6 and IL-10 levels were increased in lavage fluid obtained from infants who developed CLD. (2,12–15) Our results are consistent with these previous reports.

It has been reported that, as with intravenous dexamethasone, inhaled aerosolised corticosteroid therapy effectively decreases the need for mechanical ventilation in infants at high risk for developing CLD. (16) Other studies demonstrated that inhaled corticosteroid therapy in ventilated preterm infants was not effective in reducing the incidence of CLD. (17,18) The effect of inhaled corticosteroid therapy on CLD remains under debate. It has been reported that a 12-day course of inhaled corticosteroid therapy did not change IL-8 levels in sputum from preterm infants with CLD. (19) The results of our present study demonstrate that IL-8, TNF-α, IL-6 and IL-10 levels in sputum from infants during week 3–4 were significantly lower than those during week 1–2; however, the cytokines levels during week 1–2 were not significantly lower than those before inhaled corticosteroid therapy. It is likely that inhaled corticosteroids decrease pro-inflammatory cytokine levels in sputum from infants with CLD from 2 weeks after the start of therapy. In addition, our results revealed that levels of IL-10 (an anti-inflammatory cytokine) before the initiation of inhaled corticosteroid therapy correlated with those of TNF-α. IL-10 levels in sputum may reflect airway inflammation induced by TNF-α.

IL-8 levels were increased in samples of lung fluid from infants with CLD, (20–22) and the increase in IL-8 levels in the bronchoalveolar lavage fluid around day 10 of life in preterm ventilated infants was related to the development of CLD. (21) We have demonstrated that IL-8 levels in sputum

during week 3–4 were correlated with supplemental oxygen after extubation. The IL-8 levels in sputum during week 3–4 may determine the severity of CLD.

Oscillation is regarded as the optimal protective ventilation mode, providing small tidal volume ventilation. (23) Several previous reports have suggested that oscillation effectively prevented CLD. (24–26) Our data revealed that the ratios of IL-8 levels during week 1–2 to those before the inhaled corticosteroid therapy in infants with oscillation were significantly lower than in infants with mandate. Oscillation may be able to modulate airway inflammation of infants with CLD earlier than mandate. The ratios of IL-12p70 levels during week 1–2 to those before the inhaled corticosteroid therapy in infants with oscillation were significantly higher than in those with mandate. It has been reported that low IL-12p70 levels in tracheal aspirates were associated with more severe respiratory failure. (27) Low IL-12p70 levels in sputum were associated with neonatal airway colonisation. (27) It is likely that high IL-12p70 levels in the oscillation group could prevent airway colonisation; however, this was not investigated.

In conclusion, our results suggest that inhaled corticosteroid therapy may be associated with a decrease in pro-inflammatory cytokine levels in sputum from infants with CLD from 2 weeks after the start of therapy. The results presented here provide an impetus for large-scale studies including control subjects to explore the effect of inhaled corticosteroid therapy and oscillation on CLD.

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SUPPORTING INFORMATION

The following supporting information is available for this article:

Figure S1 Concentrations of IL-8, TNF-α, IL-1β, IL-6, IL-10 and IL-12p70 in sputum from infants with CLD before inhaled corticosteroid (ICS) therapy, and during 1–2 and 3–4 weeks after the start of therapy. Horizontal bars show the geometric mean values. Shaded areas indicate the lower detection limits. Dotted lines indicate samples from the same infants.

Figure S2 The relationship between IL-8 levels in sputum from infants with CLD during weeks 3–4 after the start of inhaled corticosteroid therapy and the supplemental oxygen period after extubation. r, Pearson's coefficient.

Table S1 Clinical characteristics of infants with CLD.

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Predictors of clinical response to interferon β1b therapy in patients with multiple sclerosis

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Background and Objective: Multiple sclerosis (MS) is a chronic and progressive inflammatory immune-mediated demyelinating disease. Interferon-β1b (IFN-β1b) treatment is effective in ameliorating relapsing-remitting MS[1], and the efficacy of IFN-β1b is potentially attributable to the immune regulatory properties of the drugs. However, some patients show a poor response to this drug. The proportion of patients who do not respond to therapy and whether particular clinical factors can predict the response to treatment are unknown. The aim of this study was to identify clinical, MRI, and biological markers predictive of clinical response to IFN-β1b therapy and to evaluate the following long-term effect of IFN-β1b treatment on chemokine receptor expression in MS patients.

Methods:
Patients: Ten patients (all women; 49 ± 11 years) included in this study had definite MS, according to McDonald criteria and were treated with IFN-β1b 8 MIU given subcutaneously every other day for at least 2 years. We compared clinical and laboratory data, including anti-aquaporin-4 (AQP4) antibody, longitudinally extensive spinal cord lesions extending over three vertebral segments, in two groups of patients - 5 of whom had relapses and the other 5 were relapse-free.
Flow cytometry: We investigated the expression of Th1-related CXCR3 and CCR5 chemokine receptors and Th2-related CCR4 chemokine receptors on T cells derived from patients undergoing IFN-β1b therapy. Venous blood was collected in heparinized tubes and analyzed within 2 h. Whole blood was labeled with directly conjugated monoclonal antibodies, according to the manufacturer's instructions, using anti-CD3 PerCP, anti-CD4 FITC, anti-CD8 FITC, anti-CXCR3 PE, anti-CCR5 PE, and anti-CCR4 PE[2]. Flow cytometric data were processed using CellQuest software.

Results: Conventional MS was more common in the no-relapse than in the relapse group and the no-relapse patients did not show longitudinally extensive spinal cord lesions from MRI. In addition, the no-relapse patients showed lower Kurtzke's expanded disability status scale (EDSS) scores, shorter disease duration, a greater number of relapses in the year prior to IFN-β1b treatment, and a markedly lower frequency of auto-immune antibodies, including anti-AQP4 antibody (Table 1). Before IFN-β1b treatment, no difference was observed in the percentage of CD4+CXCR3+ cells, CD8+CXCR3+ cells, CD4+CCR5+ cells, CD8+CCR5+ cells, CD4+CCR4+ cells, or CD8+CCR4+ cells between these groups. The percentages of CD4+CXCR3+ cells were significantly decreased after 6-24 months IFN-β1b treatment compared with the pretreatment level, while no changes for those percentages were observed in the untreated MS patients group (Figure 1A). Treatment with IFN-β1b reduced the percentage of CXCR3-expressing CD4 T cells in both relapse and non-relapse groups during the first 12 months. At 24th months after the treatment, the CXCR3 expression for non-relapse patients was still reduced. However, CXCR3 expression for relapse patients returned to the baseline level (Figure 1B). No significant changes were observed in the percentage of CCR5 or CCR4-expressing CD4 T cells between the relapse patients and the non-relapse patients.

Conclusions: Clinical findings preceding the therapy, spinal cord MRI findings or frequency of auto-immune antibodies may be the predictors of response to IFN-β1b therapy, and continuation of the decreased percentages of CD4+CXCR3+ cells may implicate long-term effectiveness to IFN-β1b treatment.

Table 1: Clinical, MRI, and biological markers in non-relapse patients and relapse patients.

	Non-relapse (n = 5)	Relapse (n = 5)	p value
Type of MS (CMS : OSMS)	4 : 1	1 : 4	0.058
Disease duration	2.4 ± 2.2	13.8 ± 11.0	0.032
EDSS score	3.0 ± 1.1	5.1 ± 1.7	0.032
Relapse rate during 1 years before IFN-β	2.8 ± 1.0	1.6 ± 0.9	0.048
ANA titer (20×2^X)	0.25 ± 0.50	3.40 ± 2.97	0.06
Serum antoantibodies	1 / 5 (0%)	4 / 5 (80%)	0.058
Anti-AQP4 antibody	1 / 5 (20%)	3 / 5 (60%)	0.20
Longitudinally extensive spinal cord lesion	0 / 5 (0%)	3 / 5 (60%)	0.038

MS; multiple sclerosis, CMS; conventional MS, OSMS; optic-spinal MS, EDSS score; Kurtzke's expanded disability status scale score, IFN-β; interferon-β, ANA; antinuclear antibody, AQP4; aquaporin 4.

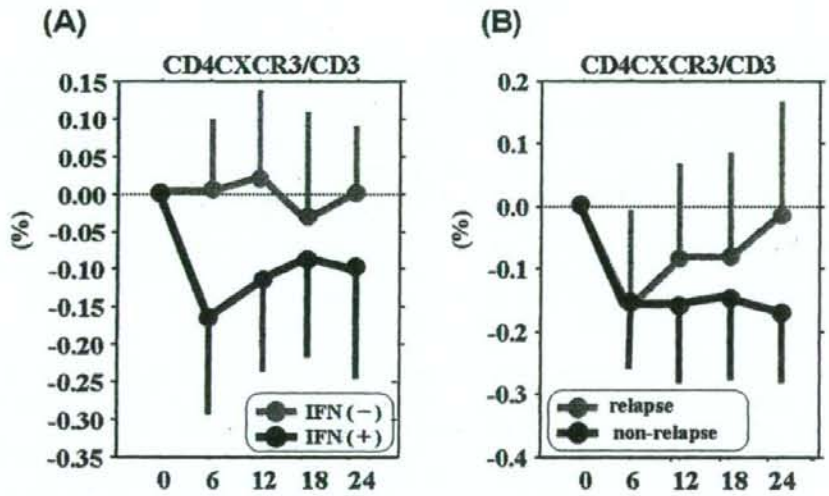

Figure 1. Changes in the percentages of CXCR3-expressing CD4 T cells during IFN-β1b treatment. (A) Comparisons between IFN-β1b treated and untreated patients. (B) Comparisons between non-relapse and relapse patients.

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Cysteinyl Leukotrienes Induce Macrophage Inflammatory Protein-1 in Human Monocytes/Macrophages

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Key Words
Leukotriene · Macrophage inflammatory protein · Mitogen-activated protein kinases · Monocytic cell line · Peripheral blood mononuclear cell · Pranlukast

Abstract

Background: Macrophage inflammatory protein-1α (MIP-1α) and MIP-1β are known for their chemotactic and pro-inflammatory effects on monocytes/macrophages which have a cysteinyl leukotriene 1 ($CysLT_1$) receptor. ***Methods:*** We examined MIP-1α and MIP-1β production stimulated by CysLTs (LTC_4, LTD_4, and LTE_4) in THP-1 cells, a human monocytic leukemia cell line, and peripheral blood mononuclear cells (PBMCs). Moreover, we examined the inhibitory effect of pranlukast, a $CysLT_1$ receptor antagonist, and inhibitors of three major mitogen-activated protein kinases (MAPK) on the induction of MIP-1α and MIP-1β production by CysLTs. ***Results:*** ELISA demonstrated that CysLTs induced MIP-1α and MIP-1β production in THP-1 cells and PBMCs. PCR demonstrated that LTD_4 increased MIP-1α and MIP-1β mRNA expressions in THP-1 cells. Pranlukast blocked MIP-1α and MIP-1β production promoted by LTD_4 in THP-1 cells and PBMCs. Moreover, an inhibitor of extracellular signal-regulated kinase (ERK) attenuated the induction of MIP-1α and MIP-1β production by LTD_4 in THP-1 cells whereas the inhibitors of c-Jun NH2-terminal kinase or p38 MAPK did not. ***Conclusion:*** CysLTs induce MIP-1α and MIP-1β production mediated by ERK via binding to the $CysLT_1$ receptor in human monocytes/macrophages.

Introduction

Cysteinyl leukotrienes (CysLTs) such as leukotriene C_4 (LTC_4), LTD_4 and LTE_4 play important roles in bronchial asthma [1–4]. CysLTs exert potent effects on tracheal muscle contraction, leukocyte trafficking, airway mucus secretion and collagen synthesis [1–4]. Normal peripheral blood leukocytes such as basophils, eosinophils, B lymphocytes and monocytes/macrophages have a $CysLT_1$ receptor [5]. However, the function of these cells, especially monocytes/macrophages, is poorly understood.

Macrophage inflammatory protein-1 (MIP-1) is a member of the β or CC subfamily of chemokines and is produced by various cells, mainly monocytes/macrophages, dendritic cells, and lymphocytes [6]. Previous studies have demonstrated that MIP-1α and MIP-1β modify the pathogenesis of asthma [6–10]. A previous paper revealed that MIP-1α production was stimulated by LTD_4 and lipopolysaccharides in alveolar macrophages of rats [11].

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We examined MIP-1α and MIP-1β production induced by CysLTs to elucidate the biological function of the $CysLT_1$ receptor of human monocytes/macrophages. Moreover, we examined MIP-1α and MIP-1β production via mitogen-activated protein kinase (MAPK).

Materials and Methods

Cell Culture and Stimulation Conditions

THP-1 cells comprising a human monocytic leukemia cell line which has a $CysLT_1$ receptor [12] were obtained from the American Type Culture Collection. The cells were maintained as stationary cultures at 37°C under a humidified 5% CO_2 atmosphere. They were grown in RPMI-1640 medium containing 10% fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Blood was drawn with informed consent from 8 healthy, medication-free volunteers. Following heparinization of the blood samples, peripheral blood mononuclear cells (PBMCs) were obtained by Histopaque 1077 (Sigma Chemical Co., St. Louis, Mo., USA) gradient centrifugation, with subsequent washing. Cells were exposed to LTC_4, LTD_4, or LTE_4 (Sigma Chemical Company). Some samples were pretreated with pranlukast, a $CysLT_1$ receptor antagonist provided by ONO Pharmaceutical Co. (Osaka, Japan), p38 MAPK inhibitor SB203580, c-Jun NH2-terminal kinase (JNK) inhibitor SP600125, and extracellular signal-regulated kinase (ERK) inhibitor PD98059 purchased from Calbiochem Corp. (San Diego, Calif., USA) for 30 min before the addition of CysLTs. Supernatant fluid was harvested for the determination of MIP-1α and MIP-1β levels both before and after the addition of CysLTs and then stored at –20°C.

Determination of the Concentrations of MIP-1α and MIP-1β

The concentrations of MIP-1α and MIP-1β in the supernatant fluid were measured with sandwich-type ELISA kits (R&D Systems, Minneapolis, Minn., USA) according to the manufacturer's instructions. The detection limits were 10 pg/ml for MIP-1α and 4.0 pg/ml for MIP-1β.

Real-Time Reverse Transcription-PCR

Real-time reverse transcription-PCR was performed to determine the mRNA levels of MIP-1α and MIP-1β in THP-1 cells subjected to the indicated treatments. Total cellular RNA was isolated using TRIzol reagent (Invitrogen, Leek, The Netherlands) according to the manufacturer's instructions. Reverse transcription was carried out with 2 μg of total RNA and Random Primers (Invitrogen) in a reaction volume of 20 μl using the SuperScript™ III RTS First-Strand Strips System (Invitrogen) following the instructions provided by the manufacturer. Real-time PCR was carried out using TaqMan Gene Expression Assays and the ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems, Foster City, Calif., USA). The PCRs were recorded in real time and analyzed using the accompanying software, Relative Quantification Program. The mRNA level of the glyceraldehyde-3-phosphate dehydrogenase housekeeping gene was also determined by real-time reverse transcription PCR in each cDNA sample to normalize the expression of the genes of interest (MIP-1α and MIP-1β).

Statistical Analysis

The values of MIP-1α and MIP-1β concentrations are expressed as the means ± SD. Statistical analysis was performed with the Wilcoxon-matched paired test, with a p value of less than 0.05 being taken as significant. Analyses and calculations were performed using SPSS-12.0 (SPSS, Inc., Chicago, Ill., USA).

Results

The concentrations of MIP-1α and MIP-1β in the culture fluid of THP-1 cells exposed to varying concentrations of LTC_4, LTD_4, and LTE_4 (10^{-6} to 10^{-11} M) for 4 h are shown in figures 1 and 2. Figure 1 shows that 10^{-6} to 10^{-9} M LTC_4 and LTD_4 significantly induced MIP-1α production in THP-1 cells ($p < 0.01$). High-dose LTE_4 (10^{-6} M) significantly induced MIP-1α production ($p < 0.05$) whereas other LTE_4 concentrations (10^{-7} to 10^{-11} M) did not. Figure 2 shows that all concentrations of LTC_4 and LTD_4 significantly induced MIP-1β production in THP-1 cells (10^{-10} and 10^{-11} M, $p < 0.05$, 10^{-6} to 10^{-9} M, $p < 0.01$). High-dose LTE_4 (10^{-6} M) significantly induced MIP-1β production ($p < 0.05$) whereas other LTE_4 concentrations (10^{-7} to 10^{-11} M) did not. Interestingly, 10^{-8} M of LTC_4 and LTD_4 induced a more significant production of MIP-1α and MIP-1β than 10^{-6} and 10^{-7} M, respectively ($p < 0.01$). MIP-1β release induced by 10^{-6} to 10^{-11} M LTC_4, 10^{-6} to 10^{-11} M LTD_4, and 10^{-6} M LTE_4 was significantly greater than that induced by MIP-1α (10^{-10} and 10^{-11} M LTC_4 and LTD_4, and 10^{-6} M LTE_4, $p < 0.05$, 10^{-6} to 10^{-9} M LTC_4 and LTD_4, $p < 0.01$). The time course of MIP-1β release was examined by incubating THP-1 cells with 10^{-8} M LTD_4 over a 24-hour period (fig. 3). MIP-1β release could be observed 4 h after the addition of LTD_4, and gradually decreased over 24 h. The time course of MIP-1α release was similar to that of MIP-1β (data not shown).

Figures 4 and 5 demonstrate MIP-1α and MIP-1β release in THP-1 cells induced by 10^{-8} M LTD_4 in the presence or absence of a 4-hour pretreatment with pranlukast, p38 MAPK inhibitor SB203580, JNK inhibitor SP600125, or ERK inhibitor PD98059. Figure 4 reveals that the production of MIP-1α induced by LTD_4 was completely inhibited by 10^{-6} M pranlukast ($p < 0.01$) and was attenuated by ERK inhibitor PD98059 ($p < 0.05$). Figure 5 shows that the production of MIP-1β induced by LTD_4 was completely inhibited by 10^{-6} M pranlukast ($p < 0.01$) and was attenuated by ERK inhibitor PD98059 ($p < 0.05$). The p38 MAPK inhibitor SB203580 or JNK inhibitor SP600125 had no effects on MIP-1α or MIP-1β production induced by LTD_4.

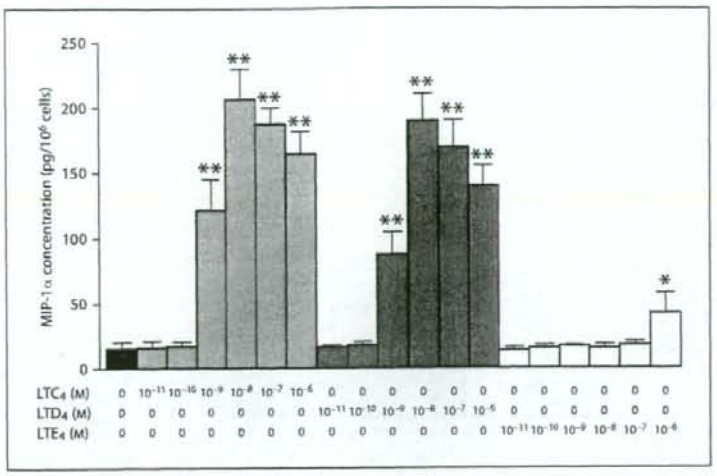


Fig. 1. MIP-1α production as measured by ELISA in THP-1 cells stimulated with LTC_4, LTD_4, or LTE_4 for 4 h. Data (n = 8) are presented as means ± 1 SD. ** $p < 0.01$, and * $p < 0.05$ compared with the culture fluid of cells not treated with LTC_4, LTD_4, or LTE_4.

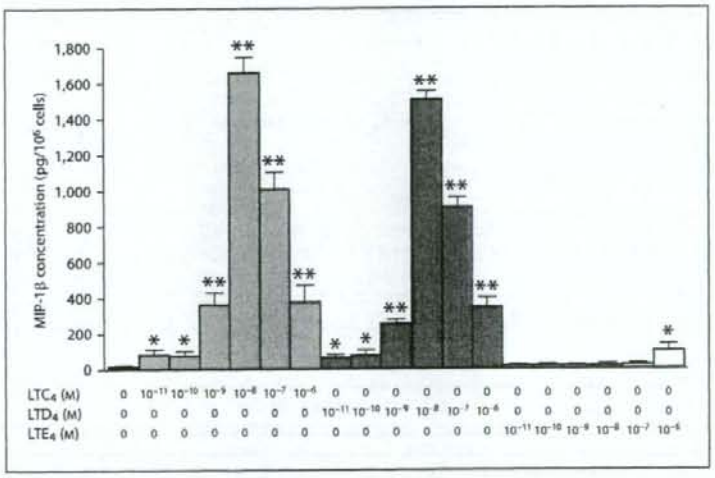


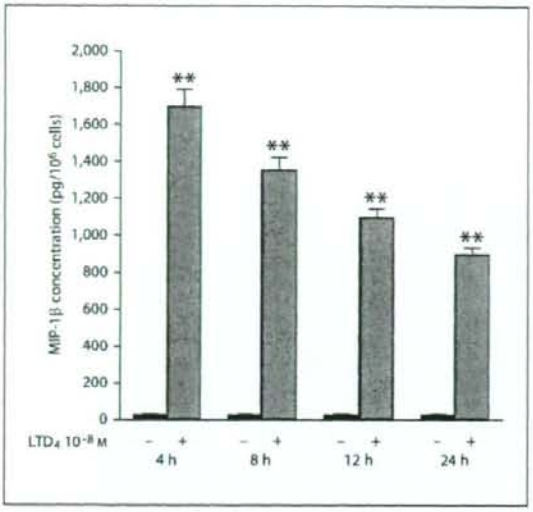
Fig. 2. MIP-1β production as measured by ELISA in THP-1 cells stimulated with LTC_4, LTD_4, or LTE_4 for 4 h. Data (n = 8) are presented as means ± 1 SD. ** $p < 0.01$, and * $p < 0.05$ compared with the culture fluid of cells not treated with LTC_4, LTD_4, or LTE_4.

Real-time PCR demonstrated that MIP-1α and MIP-1β mRNA expressions were significantly increased 4 h after the addition of 10^{-8} M LTD_4 in THP-1 cells ($p < 0.01$) (fig. 6). Moreover, pretreatment with 10^{-6} M pranlukast completely inhibited MIP-1α and MIP-1β mRNA expressions induced by treatment with LTD_4 in THP-1 cells ($p < 0.01$).

MIP-1α and MIP-1β production induced by CysLTs and the inhibitory effect of pranlukast on this production were examined by incubating PBMCs with 10^{-8} M LTD_4 for 4 h (fig. 7). LTD_4 induced significant production of MIP-1α and MIP-1β ($p < 0.01$), and pretreatment with 10^{-6} M pranlukast completely inhibited MIP-1α and MIP-1β production induced by LTD_4 in PBMCs ($p < 0.01$).

Discussion

Alveolar macrophages are the most abundant cells in the alveoli, distal air spaces and conducting airways [13]. They are activated in asthma, and activated alveolar macrophages play important roles in allergic disease of the



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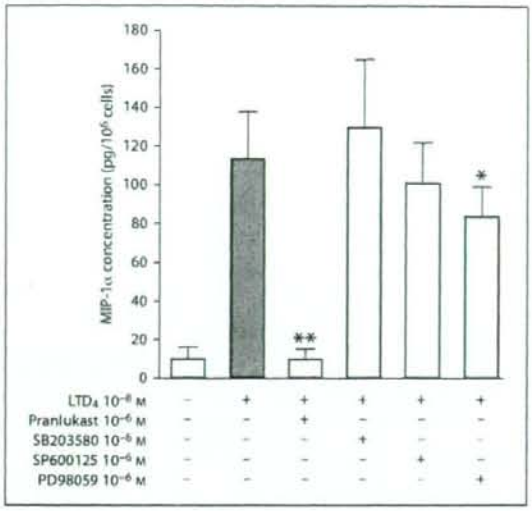

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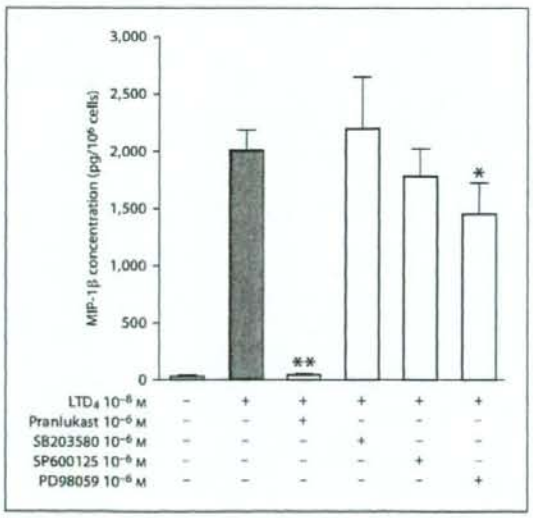
Fig. 3. Kinetics of MIP-1β production as measured by ELISA in THP-1 cells stimulated with 10^{-8} M LTD_4. Data (n = 8) are presented as means ± 1 SD. ** $p < 0.01$ compared with the culture fluid of cells not treated with LTD_4.

Fig. 4. Inhibitory effect of pranlukast, p38 MAPK inhibitor SB203580, JNK inhibitor SP600125 and/or ERK inhibitor PD98059 on MIP-1α release as measured by ELISA in THP-1 cells stimulated with 10^{-8} M LTD_4 for 4 h (cells pretreated with pranlukast, p38 MAPK inhibitor SB203580, JNK inhibitor SP600125, or ERK inhibitor PD98059 for 30 min before LTD_4 treatment). Data (n = 8) are presented as means ± 1 SD. ** $p < 0.01$, and * $p < 0.05$ compared with the culture fluid of cells treated with LTD_4 only (no pretreatment).

Fig. 5. Inhibitory effect of pranlukast, p38 MAPK inhibitor SB203580, JNK inhibitor SP600125, and/or ERK inhibitor PD98059 on MIP-1β release as measured by ELISA in THP-1 cells stimulated with 10^{-8} M LTD_4 for 4 h (cells pretreated with pranlukast, p38 MAPK inhibitor SB203580, JNK inhibitor SP600125, or ERK inhibitor PD98059 for 30 min before LTD_4 treatment). Data (n = 8) are presented as means ± 1 SD. ** $p < 0.01$, and * $p < 0.05$ compared with the culture fluid of cells treated with LTD_4 only (no pretreatment).



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lower airways, including the release of cytokines, chemokines, arachidonic acid metabolites and products of activated O_2 by direct interaction with T lymphocytes [14–17].

We investigated the functions of the $CysLT_1$ receptor in monocytes/macrophages and the effects of antagonists in prior research [18–21] and demonstrated that CysLTs induce monocyte chemoattractant protein 1 (MCP-1) and enhance the expression of tumor necrosis factor-α (TNF-α)-induced matrix metalloproteinase-9 (MMP-9) in human monocytes/macrophages [20, 21]. Moreover, we also found that $CysLT_1$ receptor antagonists inhibit nuclear factor-κB (NF-κB) activation induced by TNF-α, lipopolysaccharide-induced interleukin-6 production, MCP-1 release in the presence of CysLTs, and the enhancement of TNF-α-induced MMP-9 production by

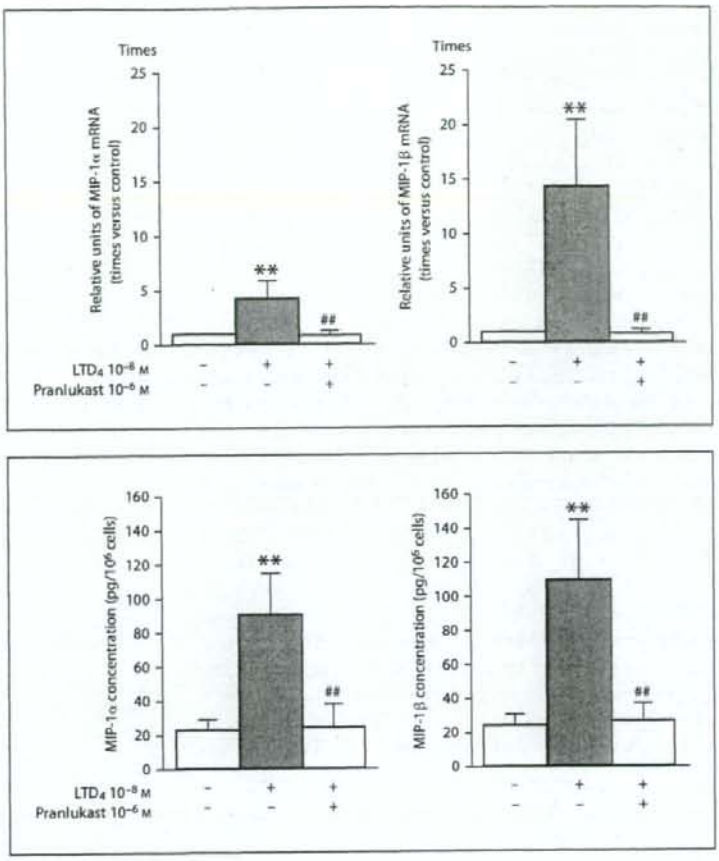

Fig. 6. MIP-1α and MIP-1β mRNA expressions and inhibitory effect of pranlukast on them as measured by real-time PCR, in THP-1 cells stimulated with 10^{-8} M LTD_4 for 4 h (cells pretreated with 10^{-6} M pranlukast for 30 min before LTD_4 treatment). Data (n = 8) are presented as means ± 1 SD. ** $p < 0.01$ compared with the culture fluid of cells not treated with LTD_4. ## $p < 0.01$ compared with cells treated with LTD_4 only (no pretreatment).

Fig. 7. MIP-1α and MIP-1β production and inhibitory effects of pranlukast on MIP-1α and MIP-1β release as measured by ELISA in PBMCs stimulated with 10^{-8} M LTD_4 for 4 h (cells pretreated with pranlukast for 30 min before LTD_4 treatment). Data (n = 8) are presented as means ± 1 SD. ** $p < 0.01$ compared with the culture fluid of cells not treated with LTD_4. ## $p < 0.01$ compared with cells treated with LTD_4 only (no pretreatment).

CysLTs in human monocytes/macrophages [18–21]. We recently demonstrated too that CysLTs induced MIP-1α and MIP-1β production and that this was inhibited by pranlukast and the ERK inhibitor. The potency of CysLTs to induce MIP-1β release was stronger than that for MIP-1α release in THP-1 cells, but not in PBMCs. We often experience a discrepancy in results between tumorous and normal cells. MIP-1α and MIP-1β chemoattract monocytes/macrophages, dendritic cells, NK cells, and T lymphocytes and modulate Th-differentiation [6]. MIP-1α chemoattracts B cells, eosinophils and basophils, degranulates eosinophils and induces histamine release from basophils and mast cells whereas MIP-1β does not [22, 23]. MIP-1α and MIP-1β are expressed and produced in various cells and airways associated with asthma [6–10]. Therefore, it is believed that MIP-1α and MIP-1β modify the pathogenesis of asthma.

Our present data suggest that MIP-1α and MIP-1β are produced via the binding of CysLTs to the $CysLT_1$ receptor because pranlukast completely inhibited MIP-1α and MIP-1β production on exposure to CysLTs. MIP-1α and MIP-1β production induced by 10^{-8} M LTC_4 and LTD_4 was significantly greater than that induced by 10^{-6} and 10^{-7} M LTC_4 and LTD_4. We previously reported that MCP-1 production induced by CysLTs in human monocytes/macrophages was not dose related [20]. Moreover, the potency of the enhancement of TNF-α-induced MMP-9 production by CysLTs was not dose related [21]. Therefore, it is likely that the potency of CysLTs was not dose related in our present study. Another hypothesis is

that higher dosages of LTC_4 and LTD_4 decreased the secretions of MIP-1α and MIP-1β because of the potential toxicity of LTC_4 and LTD_4 for THP-1 cells. A further additional assay for cell cytotoxicity will confirm this hypothesis. LTD_4 is expected to be about a 10 times more potent promoter of calcium flux at the $CysLT_1$ receptors than LTC_4 (and 100 times more than LTE_4) [24, 25]. The potencies of LTC_4 and LTD_4 regarding their induction of the MIP-1α and MIP-1β production in monocytes/macrophages may be different from those for the contraction of airway smooth muscle via the calcium flux.

In recent years, it has been revealed that several intracellular signaling molecules are related to the pathogenesis of asthma. The MAPK signaling pathway is important as one of the inflammatory pathways in asthma [26]. There are three subfamilies of mammalian MAPKs: p38 MAPK, JNK and ERK [27], which are major players in mediating a variety of signals regarding cellular functions [27]. In our present study, we focused on the MAPK signaling cascades. We revealed that the ERK inhibitor PD98059 partially blocked MIP-1α and MIP-1β release stimulated by LTD_4 whereas p38 MAPK inhibitor SB203580 or JNK inhibitor SP600125 did not. A previous paper reported that MIP-1β release induced by stem cell factor was mediated by ERK in human mast cells [28]. Our present data may support this observation. It is likely that CysLTs induce MIP-1α and MIP-1β via ERK activation in human monocytes/macrophages. The ERK signaling pathway in antigen-presenting cells may promote the initial commitment of native T-helper cells toward the Th2 phenotype [29]. Successful Th2 differentiation depends on the effectiveness of the activation of the Ras/ERK pathway mediated by the T cell antigen receptor [30]. CysLTs may accelerate Th2 differentiation via ERK activation. However, we did not investigate intracellular phosphorylations of MAPKs, and other intracellular signaling molecules and/or pathways may be related to MIP-1α and MIP-1β production promoted by CysLTs because the inhibitory effect of the ERK inhibitor was partial. A further additional study involving intracellular signaling pathways of CysLTs in monocytes/macrophages is necessary.

In conclusion, CysLTs induce MIP-1α and MIP-1β production mediated by ERK via binding to the $CysLT_1$ receptor in human monocytes/macrophages. The results presented here provide an impetus for in vivo studies including cells derived from bronchoalveolar lavage to explore the role of macrophages in the pathogenesis of bronchial asthma.

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Cerebrospinal fluid levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in subacute sclerosing panencephalitis

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Summary *Objectives:* To investigate the brain inflammation and damage in subacute sclerosing panencephalitis (SSPE), the cerebrospinal fluid (CSF) concentrations of matrix metalloproteinase-9 (MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were determined in SSPE patients.
Methods: CSF MMP-9 and TIMP-1 levels were measured in 23 patients with SSPE in Papua New Guinea by ELISA.
Results: CSF MMP-9 levels and MMP-9/TIMP-1 ratios of SSPE patients were significantly higher than controls ($p < 0.001$ and $p = 0.005$, respectively). There were no significant differences in CSF TIMP-1 levels between SSPE patients and controls.
Conclusions: Previous studies suggested that CSF MMP-9 levels reflect inflammatory damage to the brain. Our findings suggest that the MMP-9 level in CSF is an indicator of inflammatory damage to the brain in SSPE.

Introduction

Subacute sclerosing panencephalitis (SSPE) is a rare, progressive inflammatory disease of the brain caused by persistent infection with the measles virus. However, the immunological pathophysiology of SSPE is still unclear.

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A high incidence of SSPE has been previously reported in Papua New Guinea (PNG).[1] The annual incidence of SSPE in the Eastern Highlands Province (EHP) of PNG in 1997–1998 was 98 per million of population under 20 years of age, the highest ever reported.[2] This incidence was reported to range from 0.1 to 6 cases per million of population in other places.[3–5] Therefore, the incidence of SSPE in the EHP of PNG was more than ten times higher.[2]

Matrix metalloproteinases (MMPs) constitute a family of enzymes that mediate the degradation of extracellular matrix proteins.[6] MMPs are produced by a wide variety of cells, such as monocytes/macrophages, T cells, neutrophils, endothelial cells, microglia, astrocytes, and oligodendrocytes.[6,7] MMPs play important roles in normal and pathological processes, including embryogenesis, wound healing, inflammation, arthritis, cardiovascular diseases, pulmonary diseases, and cancer.[8] MMPs induce myelin break-down, proinflammatory cytokine production, and axonal damage in multiple sclerosis.[9–13] The activity of MMPs is further controlled by specific tissue inhibitors of metalloproteinases (TIMPs).[14] TIMP-1 has a high affinity for MMP-9.[15] The levels of MMP-9 and TIMP-1 and the balance between these proteins have been investigated in various central nervous system (CNS) diseases, including multiple sclerosis, acute disseminated encephalomyelitis (ADEM), influenza-associated encephalopathy, neuroblastoma, cerebral amyloid angiopathy-related hemorrhage, and acute encephalopathy following prolonged febrile seizures.[16–22] We have previously investigated serum MMP-9 and TIMP-1 levels in SSPE patients.[23] In this study, we determined cerebrospinal fluid (CSF) concentrations of MMP-9 and TIMP-1 in SSPE patients in PNG to investigate their role in the pathogenesis of SSPE. Moreover, the relationship between the data and clinical stage of SSPE (Jabbour stage) was analyzed.[24]

Patients and methods

Informed consent was obtained from the parents of the patients and controls enrolled in this study. The protocol was approved by the Medical Research Advisory Committee of PNG (MRAC No. 04/01).

Subacute sclerosing panencephalitis (SSPE)

CSF samples were obtained from 23 children with SSPE (11 males and 12 females, aged from 4 to 14 years; median, 6.8 years) at Goroka Base General Hospital, from October 1997 to April 1999 (Table 1). The criteria for the diagnosis of SSPE were (1) progressive neurological disorder, particularly mental or motor deterioration, associated with a positive history or the presence of myoclonic jerks, (2) positive CSF measles antibody titer determined by enzyme-linked immunosorbent assaying (EIA), and (3) periodic, high, amplitude, synchronous discharges in the EEG ($n = 20$). One patient was included in Jabbour stage I characterized by psychointellectual dysfunction, 16 patients in stage II characterized by convulsive and motor signs, and six patients in stage III characterized by deterioration of the state of consciousness to coma.[24] The CSF samples were stored at −70 °C.

Table 1 Clinical characteristics of SSPE patients and control subjects

	SSPE patients $N = 23$	Control subjects $N = 23$
Age in years (median, range)	6.8, 4–14	6.1, 3–13
Sex (male:female)	11:12	11:12
Jabbour stage		
Stage I	$N = 1$	
Stage II	$N = 16$	
Stage III	$N = 6$	
Final diagnosis		
Psychomotor delay		$N = 9$
Epilepsy		$N = 8$
Psychosis		$N = 3$
Myopathy		$N = 2$
Tic		$N = 1$

Control subjects

The control subjects for the CSF levels of MMP-9 and TIMP-1 were 23 afebrile and noninfectious Japanese children with neurological disorders, such as psychomotor delay, epilepsy, phychosis, etc. (eleven males and twelve females, aged from 3 to 13 years; median, 6.1 years) (Table 1). CSF samples were obtained from them on routine analysis and they all had normal CSF cell counts. There were no significant differences in age between the children with SSPE and controls (by Mann–Whitney U test).

Determination of MMP-9 and TIMP-1 concentrations

The CSF concentrations of MMP-9 and TIMP-1 were determined with sandwich-type ELISA kits (Amersham, Buckinghamshire, England), following the instructions of the manufacturer. A monoclonal coating antibody was absorbed onto polystyrene microwells to bind to MMP-9 or TIMP-1 in the samples or the standard. A horseradish peroxidase-conjugated monoclonal antibody with neutralizing activity toward MMP-9 or TIMP-1 was added to bind to MMP-9 or TIMP-1 captured by the first antibody. A substrate solution reactive with horseradish peroxidase was then added to the wells to produce a color reaction in proportion to the amount of MMP-9 or TIMP-1, and the absorbance was measured. The detection limits were 0.125 ng/ml for MMP-9 and 1.2 ng/ml for TIMP-1. The assay for MMP-9 can detect the pro- and active forms of MMP-9 (total MMP-9). In order to measure the total MMP-9 in the CSF samples, any bound MMP-9 in its pro form is activated using *p*-aminophenylmercuric acetate. The standard is pro MMP-9 which is activated in parallel for both types of sample. Active MMP-9 is detected through activation of the modified pro detection enzyme and the subsequent cleavage of its chromogenic peptide substrate.