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Biochemical and Biophysical Research Communications 324 (2004) 114–117
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The effect of long-term treatment with erythromycin on Th1 and Th2 cytokines in diffuse panbronchiolitis

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Received 1 September 2004
Available online 21 September 2004

Abstract

Diffuse panbronchiolitis (DPB) is a chronic progressive disease of the respiratory bronchioles, and has been improved by low-dose, long-term erythromycin (EMC) treatment. The therapeutic benefits may be derived from its anti-inflammatory and immunomodulatory properties rather than antimicrobial effect. However, there are few studies about the mechanism of immunomodulation by EMC treatment for patient with DPB. In this study, we quantified the changes of Th1 and Th2 cytokines in the bronchoalveolar lavage (BAL) fluid from patients with DPB after long term treatment with EMC. After the EMC treatment, a significant reduction in the number of lymphocytes was observed, and the CD4/CD8 ratio was elevated as well. The IL-2 and IFN-γ levels in the BAL fluid were significantly decreased and the IL-4, IL-5, and IL-13 levels were significantly increased after EMC treatment. Our results suggest that the therapeutic benefits of long-term EMC treatment may be partially due to the immune system's shift from Th1 to Th2 cytokine production.

Keywords: Erythromycin; T cell; Cytokine; Diffuse panbronchiolitis

Diffuse panbronchiolitis (DPB) is characterized clinically by progressive airflow limitation and recurrent respiratory infection, and pathologically by chronic inflammation that is predominantly localized in the respiratory bronchioles with infiltration of inflammatory cells, such as neutrophils, lymphocytes, and plasma cells [1,2]. Its incidence has been mainly reported in Japan, Korea, and China, and it has rarely been reported outside the Far East Asia [2,3]. However, cases of DPB have been recently recognized in the United States, Latin America, and Europe, raising concerns that it might be underdiagnosed in these populations [4,5]. If DPB is left untreated, the patient's condition deteriorates rapidly and without treatment, the outcome is fatal [1,6]. However, this dismal outcome has been improved by the introduction of long-term, low dose macrolide treatment [7,8]. Several investigators have sought to establish why the majority of patients with DPB improve on macrolide treatment when they do not appear to have a bacterial infection, and it has been suggested that the efficacy might be derived from anti-inflammatory and immunomodulatory activities [9]. Subsequent studies have shown that macrolides suppress the production of several pro-inflammatory cytokines produced by bronchial epithelial cells, neutrophils, lymphocytes, and monocytes [10–12]. Previous studies have suggested that lymphocytes are the important cellular components of bronchial inflammation in DPB [13]. However, there is no study available about the effect of long-term treatment with erythromycin (EMC) on Th1 and Th2 cytokines in DPB. Thus, the purposes of the present study are to investigate the T-cell subsets and to evaluate the changes of the Th1 and Th2 cytokines in the bronchoalveolar lavage (BAL) fluid of DPB patients before and after 6 months of EMC treatment.

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doi:10.1016/j.bbrc.2004.09.018

Materials and methods

Subject population. We examined 14 patients who fulfilled all clinical criteria for DPB as published by the Japanese Ministry of Health and Welfare in 1995, or histopathologically diagnosed as having DPB by surgical lung biopsy. The clinical criteria for DPB were as follows: (1) symptoms of chronic cough, sputum, and dyspnea on exertion; (2) physical signs with rales; (3) diffuse disseminated fine nodular shadows shown mainly in the lower lung fields on chest radiograph or computed tomography; (4) pulmonary function tests and blood gas analysis (FEV_1 percentage predicted of <70% and $PaO_2 < 80$ mm Hg); (5) elevated titers of cold hemagglutinin (64× or higher); and (6) history or coexistence of chronic sinusitis. In 11 of the 14 patients, the disease was histologically confirmed by open lung biopsy specimens, and the remaining 3 patients were diagnosed clinically. None of them had a clinically pulmonary infection within 1 month prior to BAL. However, BAL fluid culture demonstrated *Pseudomonas aeruginosa* in 7 patients. All patients received 250 mg of EMC orally twice daily for at least 6 months. None received other antibiotics or corticosteroids during the course of the study. BAL was performed before and after 6 months EMC treatment.

Bronchoalveolar lavage. The patients were premedicated intramuscularly with atropine (0.5 mg), and 4% lidocaine was used as a local anesthetic. BAL was performed using a flexible fiberoptic bronchoscope (PENTAX EB 1830T2; PENTAX, Tokyo, Japan) with monitoring of heart rate and transcutaneous oxygen saturation throughout the procedure. The bronchoscope was securely wedged into the subsegmental bronchus of the right middle lobe, and 150 mL normal saline at body temperature was infused in three 50 mL aliquots. The fluid was gently aspirated immediately after each infusion using a wall suction pressure of 80–100 mm Hg. The BAL fluid aspirated after each instillation was pooled together into a single specimen and immediately placed on ice.

Processing and analysis of the BAL fluid. The pooled BAL fluid was placed into two aliquots. One aliquot of the pooled BAL fluid was submitted for viral and bacterial cultures to the hospital microbiology department. The remainder of the pooled BAL fluid was taken to the laboratory for analysis of the cellular and fluid fractions. The fluid was then centrifuged at 400*g* for 10 min at 4 °C, and the supernatants were kept at −70 °C until use. The total cell numbers were counted with a hemocytometer (Weber, Teddington, United Kingdom). Smears of BAL cells were prepared with a cytospin. The smears were stained with Diff-Quik solution (Dade Diagnostics of P.R. Aguada, PR) in order to examine the cell differentials. Two independent, blinded investigators counted the cells using a microscope. Approximately 400 cells were counted in each of four different random locations, and the inter-investigator variation was <5%. The mean number from the two investigators was used to estimate the cell differentials.

Flow cytometric anlaysis. The unseparated BAL cells (1×10^5 cells) were stained by through simultaneous additions of fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated antibodies specific for cell-surface markers (Becton–Dickinson Immunocytometry Systems, San Jose, CA, USA). The stained cells were analyzed on a FACSscan flow cytometer (FACS Division, Becton–Dickinson), and a Consort 30 computer system (Becton–Dickinson) was used for data acquisition and analysis.

Cytokine assays. Levels of IL-2, IL-4, IL-5, IL-13, and IFN-γ were quantified in the supernatants of the BAL fluids by using a commercial ELISA kit according to the manufacturer's protocol (Endogen, Woburn, Mass). The kits were able to detect concentrations as low as 2 pg/mL.

Statistical analysis. Data were expressed as means ± SD. A paired *t* test or a Wilcoxon signed-rank test was used to compare the cell count and cytokine levels in the BAL fluids before and after EMC treatment. A *p* value of 0.05 indicated statistical significance.

Results

Subject characteristics

Fourteen patients with DPB (9 men and 5 women; mean age, 45.50 ± 17.31 years; 2 smokers and 12 nonsmokers) were completed the study. The arterial blood gas analysis showed a decreased PaO_2 with a mean value of 72.40 ± 5.60 mm Hg. The mean value of forced expiratory volume in 1 s (FEV_1) was 65.50 ± 3.46%.

BAL cell profiles

Each of the neutrophil and macrophage percentages in the BAL fluids of the 14 patients with DPB was compared before and after EMC treatment. The percentage of neutrophils in the BAL fluids was significantly decreased after EMC treatment from 68.27 ± 16.74% to 20.36 ± 19.07% ($p < 0.01$), and the percentage of macrophages was significantly increased from 28.28 ± 16.17% to 68.61 ± 20.94% ($p < 0.01$; Fig. 1).

CD4+ and CD8+ T lymphocytes in BAL

The T-cell subsets in the BAL fluid were compared before and after EMC treatment. The percentage of CD4+ and CD8+ T lymphocytes in the BAL fluid was significantly decreased after EMC treatment as compared with before the treatment ($p < 0.05$ for CD4+, $p < 0.01$ for CD8+; Fig. 2A). However, the CD4+/CD8+ T lymphocyte ratio in the BAL fluid after EMC treatment was significantly higher than before the treatment ($p < 0.01$; Fig. 2B).

Cytokines in BAL

The levels of IL-2, IL-4, IL-5, IL-13, and IFN-γ in the BAL fluid as analyzed by ELISA were compared before and after EMC treatment (Fig. 3). The concentration of IL-2 in the BAL fluid after EMC treatment was significantly lower than before the treatment

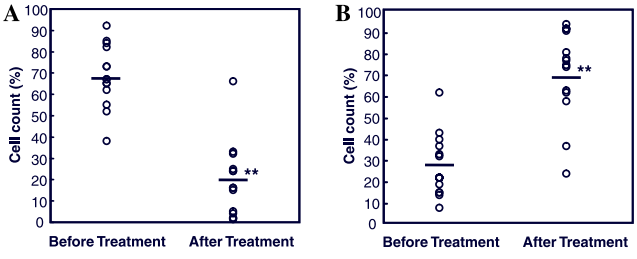

Fig. 1. BAL fluid cell profiles. (A) BAL fluids showed a significantly lower percentage of neutrophils after treatment as compared with before treatment. (B) BAL fluids showed a significantly higher percentage of macrophages after treatment as compared with before treatment. (**$p < 0.01$).

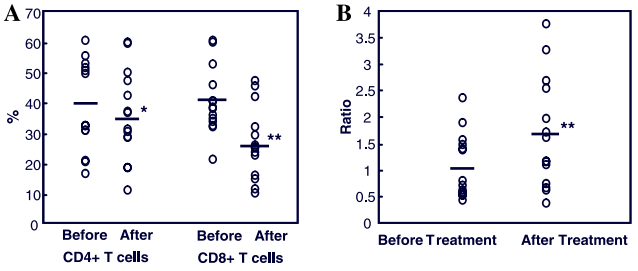

Fig. 2. CD4+ and CD8+ T lymphocytes in the BAL fluids. (A) The percentages of CD4+ and CD8+ T lymphocytes in the BAL fluids were significant lower after treatment than before treatment. (B) The CD4+/CD8+ T lymphocyte ratio in the BAL fluids was significantly higher after treatment as compared with before treatment. (*$p < 0.05$, **$p < 0.01$).

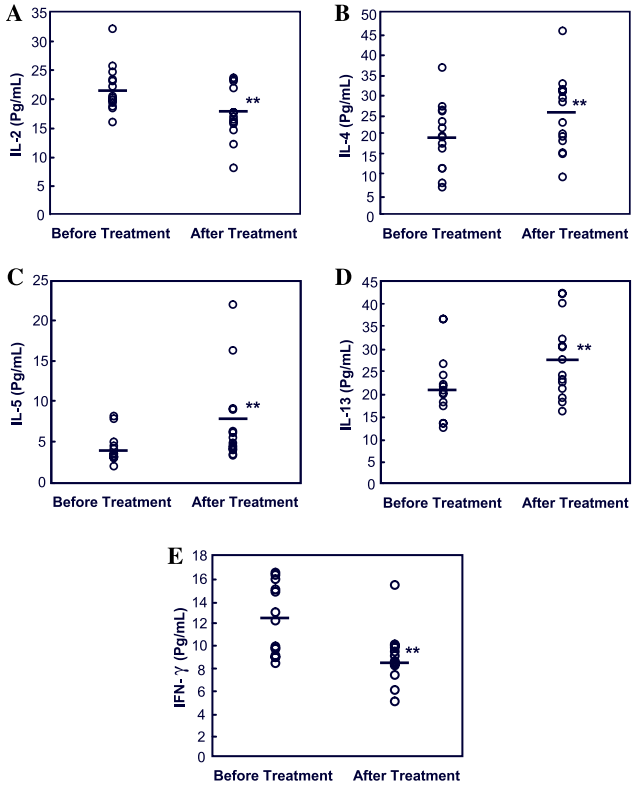

Fig. 3. The levels of IL-2, IL-4, IL-5, IL-13, and IFN-γ in the BAL fluids were compared before and after EM treatment. IL-2 (A) and IFN-γ (E) concentrations in the BAL fluid were significantly lower after treatment than before treatment. IL-4 (B), IL-5 (C), and IL-13 (D) concentrations in the BAL fluid were significantly higher after treatment as compared with before treatment. (**$p < 0.01$).

(22.21 ± 4.50 pg/mL vs. 16.41 ± 4.15 pg/mL, respectively; $p < 0.01$). However, the concentrations of IL-4, IL-5, and IL-13 in the BAL fluid after EMC treatment were significantly higher than before the treatment ($p < 0.01$). The concentration of IFN-γ in the BAL fluid after EMC treatment was significantly lower than before the treatment (12.62 ± 3.04 pg/mL vs. 8.66 ± 2.41 pg/mL, respectively; $p < 0.01$).

Discussion

DPB is a pulmonary disease characterized by chronic inflammation of the respiratory bronchioles and chronic infiltration of inflammatory cells in the lungs. The natural history of DPB includes the development of diffuse bronchiectasis, progressive respiratory failure leading to cor pulmonale, and ultimately death. Nearly 50% of the untreated patients have died within five years of diagnosis and for a long time, the long-term prognosis of DPB was poor. However, this once dismal outcome has been improved by the introduction of long-term treatment with low dose macrolide [8,9]. While the exact mechanisms are unknown, the anti-inflammatory rather than antimicrobial properties of macrolides seem to be responsible for the beneficial effects in patients with DPB. Indeed, macrolides may affect several components of host pulmonary defense system. In vitro studies and studies using animal models have generated data to support the inhibitory effects of macrolides on neutrophil influx, on several of the neutrophil cell functions, and on cellular chemotactic activity. In numerous in vitro and ex vivo studies, it has been shown that macrolides inhibit oxidant production and promote neutrophil cell degradation [14–17]. Additionally, in vitro and ex vivo studies have clearly shown that macrolides can influence the cytokine production of several cell types [18–20]. In the clinical studies that have focused on the cytokine levels in lung fluids, long-term macrolide therapy has resulted in reduced IL-1β and IL-8 levels in patients with DPB [18,21]. However, the exact mechanisms responsible for the benefits of long-term macrolide therapy for DPB are unclear.

What are the functions of lymphocytes in DPB, and what is the effect of macrolide therapy on T cell cytokines in DPB? The activation of CD8+ T cells in the airway lumen has been detected in DPB patients, which suggests that lymphocytes are important cellular component of bronchial inflammation in DPB [13]. In patients with DPB, there were more lymphocytes and reduced CD4+/CD8+ cell ratios compared with in bronchiectasis patients and healthy controls. After patients received macrolide therapy for 2–6 months, there was a significant reduction in the number of lymphocytes and activated CD8+ cells, and an elevation in the CD4+/CD8+ ratio in BAL fluid from DPB patients [13]. Our results were very similar to these findings. The percentages of neutrophils and CD4+ T cell in the BAL fluid after long-term EMC therapy were significantly lower than before therapy, and the CD4+/CD8+ ratio after therapy was significantly higher than before therapy. These findings were already known from previous studies, but there is no study available about the effects of long-term EMC treatment on the CD4+ T cell cytokines in DPB patients.

The two major subsets of CD4+ T helper cells, Th1 and Th2, have different patterns of cytokine production

and different roles in immune responses [22]. Th1 cells secrete the cytokines IFN-γ, IL-2, TNF-β, and TNF-α, while Th2 cells secrete IL-4, IL-5, IL-6, IL-10, and IL-13. Of these cytokines, we quantified the levels of IL-2 and IFN-γ for Th1 cells and IL-4, IL-5, and IL-13 for Th2 cells by ELISA. Th1 cytokines in the BAL fluid were significantly lower concentration after treatment than before treatment, and the Th2 cytokines in the BAL fluid were significantly higher concentration after treatment than before treatment. Our findings suggest that the long-term, low-dose EMC treatment for patients with DPB affects the neutrophil accumulation and the Th1 and Th2 cytokine-mediated immune pathways, and the therapeutic benefits may be partially due to a shift in the immune systems from Th1 to Th2 cytokines.

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