Bronchoalveolar lavage cell findings in three types of eosinophilic pneumonia: acute, chronic and drug-induced eosinophilic pneumonia

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There are clinically different types of eosinophilic pneumonia (EP) but no study to date has compared pulmonary inflammatory cells between different types of EP, such as acute eosinophilic pneumonia (AEP), chronic eosinophilic pneumonia (CEP) and drug-induced eosinophilic pneumonia (drug-EP). The present study compared bronchoalveolar lavage fluid (BALF) cell findings to elucidate whether the profiles of the pulmonary inflammatory cells were different among the three types of EP.

Clinical records of 28 patients with EP, consisting of eight AEP patients, 10 CEP patients and 10 drug-EP patients, were examined retrospectively. The differential cell counts, the $CD4+/CD8+$ ratio of lymphocytes, the percentage of HLA-DR+ in CD4+ and CD8+ lymphocytes, and the mean number of nuclear segmentations in eosinophils in BALF were compared among the three types of EP.

The numbers of total cells, lymphocytes, neutrophils and eosinophils in BALF from patients with AEP were increased compared with those from normal subjects, and patients with CEP and drug-EP. The $CD4+/CD8+$ ratio of the BALF lymphocytes in patients with AEP, which exceeded 1.0 in all patients, was significantly higher than that in normal subjects. The percentages of HLA-DR+ cells in CD8+ lymphocytes in BALF from patients with CEP were significantly higher than those from patients with AEP and drug-EP. There was no significant difference in the mean number of nuclear segmentations in eosinophils in BALF among the three types of EP.

The BALF cell findings in patients with EP showed some characteristics in accordance with type of EP. It is suggested that pulmonary neutrophils and lymphocytes, rather than eosinophils, may be related to the pathogenesis of the different types of EP.

Introduction

Eosinophilic pneumonia (EP) has been defined as pulmonary infiltration of eosinophils independently of peripheral blood eosinophilia. This pulmonary disorder includes clinically different types of EP and different causes (1-6). Both acute eosinophilic pneumonia (AEP) (7) and chronic eosinophilic pneumonia (CEP) (8) have been described as idiopathic EP (5). There are some reports concerning the comparison of clinical features between AEP and CEP (9,10). However, the differences in the profiles of pulmonary inflammatory cells between the two diseases remains unknown. Analysis of bronchoalveolar lavage fluid (BALF) cells is fundamentally important in diagnosis of EP. As it is considered that the findings of BALF cells reflect the inflammatory processes of the lungs, it is suggested that the different types of EP may have distinctive profiles of BALF cells. Drug-induced EP (drug-EP), which has a specific aetiology, is common. The authors considered that it is important to compare idiopathic EP with aetiology-proven EP. The present study was conducted to elucidate the differences of BALF cell findings among AEP, CEP and drug-EP.

Subjects and Methods

SUBJECTS

Twenty-eight patients with EP, consisting of eight patients with AEP (seven men and one woman, mean age 24.9 ± 9.8 years, all smokers), 10 patients with CEP (seven men and three women, mean age 38.6 ± 19.6 years, five smokers and five non-smokers) and 10 patients with drug-EP (five men and five women, mean age 42.3 ± 19.9 years, four smokers and six non-smokers), were included in this study. Each patient was diagnosed and treated at Kanazawa University Hospital or its related hospitals between 1989 and 1995.
Five of the patients with AEP (11), and three of the patients with drug-EP (12,13) were included in the authors' previous reports. Each patient with AEP met the following diagnostic criteria: acute febrile illness of less than 7 days duration; hypoxaemia; diffuse pulmonary infiltrates on chest X-ray films; increased percentage of eosinophils in BALF; absence of possible causes; and either spontaneous improvement or prompt response to corticosteroid therapy with no relapse. Five patients were treated with corticosteroids. None of them had a history of bronchial asthma. The diagnosis of CEP was made based on the following diagnostic criteria: typical clinical features consistent with CEP (5) (fever, malaise, night sweats, weight loss, anorexia, cough, sputum); dense and multiple foci of consolidation in the peripheral lung fields on chest X-ray films; increased percentage of eosinophils in BALF; absence of possible causes; and prompt response to corticosteroid therapy. All patients were treated with corticosteroids. Five patients had bronchial asthma. Attention was paid to the differential diagnosis of allergic bronchopulmonary aspergillosis, especially in patients complicated with bronchial asthma; it was ruled out by serological and sputum examinations and skin test. Five patients had relapse of their symptoms and chest radiographic shadows. It is reported that the classification of CEP and AEP by chest radiographic findings fully coincides with that by the clinical course (10). Therefore, the authors attached importance to chest radiographic findings. The patients with drug-EP satisfied the following diagnostic criteria: fever or some respiratory symptoms, diffuse pulmonary infiltrates on chest X-ray films; increased percentage of eosinophils in BALF; prompt improvement after cessation of the causative drug; absence of other possible causes; positive reaction in lymphocyte stimulation assays; or recurrence of the symptoms with the drug challenge. The causative drugs were minocycline hydrochloride in five patients, and ofloxacin, piperacillin sodium, cefixime, cefodizime and ofloxacin, each in one patient. One patients with drug-EP had bronchial asthma. The laboratory data in the patients with EP on admission were as following (mean ± standard deviation). AEP: WBC 16 688.0 ± 4573.7 cells µl⁻¹, eosinophils 6.0 ± 14.9%, CRP 10.6 ± 4.0 mg dl⁻¹, IgE 1352.0 ± 986.8 IU ml⁻¹, PaO₂ 91.1 ± 17.8 mmHg; CEP: WBC 7533.0 ± 2220.0 cells µl⁻¹, eosinophils 15.1 ± 16.9%, CRP 3.6 ± 2.2 mg dl⁻¹, IgE 398.0 ± 646.0 IU ml⁻¹, PaO₂ 74.9 ± 12.3 mmHg; drug-EP: WBC 9562.5 ± 3574.9 cells µl⁻¹, eosinophils 16.9 ± 20.0%, CRP 4.1 ± 6.7 mg dl⁻¹, IgE 597.0 ± 649.8 IU ml⁻¹, PaO₂ 75.0 ± 11.4 mmHg.

As control, the BALF obtained from 10 normal subjects (nine men and one woman, mean age 50.5 ± 17.0 years, seven smokers and three non-smokers) was used.

**DIFFERENTIAL CELL COUNTS IN BALF**

After part of the BALF was diluted with an equal volume of Türk solution, cells were counted in a Bürker chamber. After the remaining BALF was passed through a double layer of Dacron net, cells were pelleted at 1500 rpm for 10 min and resuspended in 10 ml of RPMI-1640 (Grand Island Biological Co., Grand Island, NY, U.S.A.). The fluid was resuspended to a concentration of 2 x 10⁶ cells ml⁻¹. Smears for differential counts were prepared by cytocentrifugation (Cytospin 2, Shandon Southern Instruments, Sewickley, U.S.A.) at 800 rpm for 10 min. After staining with May-Grünwald-Giemsa, differential cell count was done in 300 cells.

**LYMPHOCYTE SUBSETS**

Each cell suspension was adjusted to a concentration of 2 x 10⁷ cells ml⁻¹. For assay, 10 µl of the monoclonal antibody was added at the proper dilution to 100 µl of the cell suspension. Cells were incubated at 4°C for 30 min, washed twice in phosphate-buffered saline, and then resuspended in 0.3 ml of RPMI-1640. They were then analysed for CD4⁺, CD8⁺, CD4⁺ and human leucocyte antigen-DR (HLA-DR)+ and CD8⁺ and HLA-DR+ lymphocytes, using a flow cytomter (FACScan, Becton Dickinsonson, Mountain View, CA, U.S.A.). Monoclonal antibodies that recognized CD8 [fluorescein isothiocyanate (FITC)-conjugated Leu2], CD4 (FITC-conjugated Leu3) and HLA-DR [phycoerythrin (PE)-conjugated HLA-DR] were provided by Becton Dickinsonson. In each analysis, cells stained by FITC- and PE-conjugated non-reactive mouse IgG (Becton Dickinsonson) were employed as negative control. Flow cytometric analyses were processed by SimulSET software in a FACScan. Appropriate fractions of lymphocytes were selected by gating on two-dimension display, on forward scatter and side scatter.

**NUCLEAR SEGMENTATION OF EOSINOPHILS IN BALF**

Nuclear segmentation of 200 BALF eosinophils was counted on slides prepared by cytocentrifugation (Cytospin 2, Shandon Southern Instruments, Sewickley, U.S.A.) at 800 rpm for 10 min and stained with May-Grünwald-Giemsa. The mean number of nuclear segmentations per eosinophil was calculated in each subject.

**STATISTICAL ANALYSIS**

Data were expressed as means ± standard deviation (SD) of the mean. Unpaired t-test was used to compare the BALF cell findings between any pairs of normal subjects and patients with AEP, CEP and drug-EP. A level of P<0.05 was considered statistically significant.

**BRONCHOALVEOLAR LAVAGE**

Bronchoalveolar lavage was performed in the acute phase without corticosteroid therapy. It was carried out in the lobe with shadow using three 50 ml aliquots of physiologic saline solution. A part of the recovered fluid was used for bacteriologic culture and cytological examination.
Fig. 1. Absolute number of differential cells in bronchoalveolar lavage fluid (BALF) from patients with acute eosinophilic pneumonia (AEP), chronic eosinophilic pneumonia (CEP) and drug-induced eosinophilic pneumonia (drug-EP). Each open square with vertical bar represents mean ± SD. *P<0.05, **P<0.01, ***P<0.001 compared with normal subjects.
Results

DIFFERENTIAL CELL COUNTS IN BALF

The absolute numbers of BALF cells are shown in Fig. 1. The number of total cells in patients with AEP, CEP and drug-EP were significantly increased compared with that in normal subjects. The number of lymphocytes in patients with AEP and CEP were significantly increased compared with that in normal subjects. The number of neutrophils in patients with AEP was significantly increased compared with that in normal subjects. The numbers of total cells, lymphocytes, neutrophils and eosinophils in patients with AEP were significantly higher than those in patients with CEP or drug-EP, respectively.

CD4+/CD8+ RATIOS OF LYMPHOCYTES IN BALF

The CD4+/CD8+ ratios of lymphocytes in BALF and peripheral blood (PB) are shown in Fig. 2. The CD4+/CD8+ ratio in the BALF from patients with AEP (2.3 ± 0.8) was significantly higher than that from normal subjects (1.4 ± 0.7). The CD4+/CD8+ ratio in patients with CEP or drug-EP did not differ from that in normal subjects. The CD4+/CD8+ ratio in BALF from patients with AEP was significantly higher than that from patients with drug-EP.

The CD4+/CD8+ ratio of lymphocytes in PB was not different among patients with AEP, CEP and drug-EP and normal subjects.

PERCENTAGE OF HLA-DR+ IN CD4+ AND CD8+ LYMPHOCYTES IN BALF

Percentages of HLA-DR+ in CD4+ and CD8+ lymphocytes in BALF and PB are shown in Fig. 3. Both the percentages of HLA-DR+ in CD4+ and CD8+ cells in BALF from patients with CEP were significantly increased compared with those from normal subjects. The percentage of HLA-DR+ in CD4+ cells in BALF from patients with CEP was significantly higher than that from patient with AEP and drug-EP.

The percentage of HLA-DR+ in CD4+ or CD8+ lymphocytes in PB did not differ among patients with AEP, CEP and drug-EP and normal subjects.

NUCLEAR SEGMENTATION OF EOSINOPHILS IN BALF

Mean numbers of nuclear segmentations in eosinophils in BALF from patients with AEP, CEP and drug-EP are shown in Fig. 4. There were no significant differences in the number of nuclear segmentations among the three patient groups.

Discussion

The present study compared the profiles of pulmonary inflammatory cells among AEP, CEP and drug-EP. In the results, the numbers of total cells, eosinophils and lymphocytes in BALF from patients with AEP, CEP and drug-EP were increased compared with those from normal subjects.
normal subjects. The authors consider that the increases of eosinophils and lymphocytes in the lungs are fundamental common profiles in all three types of EP. However, the number of neutrophils in BALF from patients with AEP was increased significantly compared with those from normal subjects and patients with CEP and drug-EP. In addition, the numbers of eosinophils and lymphocytes were also increased significantly in patients with AEP compared with CEP and drug-EP. Therefore, the inflammatory cell profile in the lungs of patients with AEP may be characterized by the intensive infiltration, especially the increase of neutrophils. Clinically, most of the patients with AEP have severe illness. Indeed, in this study, the illness was most severe in the patients with AEP. It is suggested that the degree of inflammatory cell infiltration, especially neutrophils, in the lungs is linked to the clinical condition in the patients with EP. There is no report showing the increase of eosinophils and neutrophils in BALF from AEP compared with those from CEP. In the four patients with AEP reported by Allen et al. (7), the percentage of neutrophils in BALF on admission was increased in three patients, and the neutrophil percentage was decreased during the recovery phase in the three patients. So, the present authors suspect that the discrepancy of BAL neutrophilia in AEP...
In conclusion, the BALF cell findings in patients with EP showed some distinctive characteristics in accordance with the type of EP. It is suggested that neutrophils and lymphocytes rather than eosinophils in the lungs may be related to the pathogenesis of each type of EP. Consequently, it is recommended that the function of BALF cells should be analysed in accordance with each type of EP in future studies.

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