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 lomefloxacin hydrochloride, piperacillin sodium, cefixime, cefodizime and ofloxacin, each in one patient. One patient with drug-EP had bronchial asthma. The laboratory data in the patients with EP on admission were as following (mean ± standard deviation). AEP: WBC 16688 ± 45737 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 41.1 ± 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 599.1 ± 640.8 IU ml−1, PaO2 750 ± 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 C., Grand Island, NY, U.S.A.). The fluid was resuspended to a concentration of 2 × 105 cells ml−1. Smears for differential counts were prepared by cytocentrifugation (Cytospin 2, Shandon Southern Instruments, Sewickleyo, 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 × 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 leukocyte antigen-DR (HLA-DR)+ and CD8+ and HLA-DR+ lymphocytes, using a flow cytometer (FACScan, Becton Dickinson, 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 Dickinson. In each analysis, cells stained by FITC- and PE-conjugated non-reactive mouse IgG (Becton Dickinson) 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, Sewickleyo, U.S.A.) at 800 rpm for 10 min and stained with May-Grünwald-Giemsa. The mean number of nuclear segmentation 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.
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 CD8+ 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.
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
may be due to the difference of the disease phase (acute vs. recovery) when BAL was performed. Otherwise, as all of the patients with AEP were smokers, it may be claimed that smoking affects the BALF cell findings. There was the same tendency when the BALF cell findings in the smoking patients with CEP and drug-EP were compared with those in AEP (data not shown). On the other hand, the authors also suspect that smoking in young-aged men and women may be related to AEP, because most of the patients with AEP studied were young smokers with a little experience of smoking. The patients with AEP described in previous reports are also comparatively young, but there is insufficient information concerning their smoking state. Future studies to clarify the association between smoking and AEP are needed.

The CD4+/CD8+ ratio of lymphocytes in the BALF from patients with AEP was increased compared with those from normal subjects and patients with CEP and drug-EP. In addition, although there was no certain tendency in the CD4+/CD8+ ratio in the patients with CEP or drug-EP, the ratio exceeded 1:0 in all patients with AEP. It is suspected that a variety of drugs and/or different types of allergic reactions may be responsible for the indefinite CD4+/CD8+ ratio of BALF lymphocytes in drug-EP. On the other hand, AEP and CEP are idiopathic or aetiology unknown. It is characteristic of summer-type hypersensitivity pneumonitis that the CD4+/CD8+ ratio of BALF lymphocytes are decreased below 1:0 (14). From these findings, it is suspected that the uniform increase in the CD4+/CD8+ ratios of BALF cells in AEP indicates an antigen and/or allergic reaction as the aetiology.

Next the percentage of HLA-DR+ in CD8+ lymphocytes in the BALF from patients with CEP was significantly greater than that from patients with AEP and drug-EP, suggesting that activated lymphocytes, especially activated suppressor T-cells, in BALF from patients with CEP are increased. Although the number of lymphocytes in BALF from patients with CEP was lower than that from patients with AEP, the lymphocytes in BALF from patients with CEP may be characterized by the increased activation of pulmonary suppressor T-cells. In addition, the percentage of HLA-DR+ in CD8+ lymphocytes in the peripheral blood from patients with CEP and drug-EP tended to be increased compared with that in normal subjects and patients with AEP. Causative drugs arrive at the lungs through the venous route in drug-EP, resulting in systemic immunologic reactions such as the increased percentage of HLA-DR+ in CD8+ lymphocytes in the peripheral blood. There was no difference in lymphocyte subset in the peripheral blood between the patients with AEP and normal subjects, suggesting that a causative antigen may arrive at the lungs through the respiratory tract but not through the venous route, or allergic reaction may be localized in the lungs in AEP.

Chihara et al. (15) showed hypodensity and nuclear hypersegmentation of pulmonary eosinophils in EP. In addition, they reported that some chemo-attractants and cytokines could induce nuclear hypersegmentation of eosinophils (16). Previously, the present authors have observed that the changes in mean number of nuclear segmentations of eosinophils in BALF were closely linked with the disease activity of EP (unpubl. data). It is speculated that this phenomenon may be associated with the increase of some mediators and/or cytokines which activate eosinophils in the lungs. The present study showed that there were no significant differences in the mean number of nuclear segmentations in eosinophils in BALF among AEP, CEP and drug-EP. Therefore, it is thought that the nuclear hypersegmentation of eosinophils in BALF, one of the indicators for activated eosinophils, is observed commonly regardless of different types of EP. It is suspected that there may be no difference in participating mediators and/or cytokines activating eosinophils in the lungs among the three types of EP.

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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