Plasma Cells in the Bronchoalveolar Lavage Fluid of a Patient with Eosinophilic Pneumonia*

Morphologic Proof of Local Production of Antibodies

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We describe a case of eosinophilic pneumonia in which plasma cells appeared in bronchoalveolar lavage fluid (BAL). A 49-year-old man presented with wheezing, lung infiltrates, peripheral eosinophilia, and extremely high IgE levels in serum and BAL. A differential count of BALF revealed 56.6 percent lymphocytes and 1.3 percent plasma cells. The appearance of plasma cells suggests local maturation of B cells and represents a morphologic proof of local production of immunoglobulins. The increased number of lymphocytes suggests their role in B cell differentiation via the lymphokine network.

Since its introduction, bronchoalveolar lavage (BAL), although still experimental, has been shown to be a simple, noninvasive procedure with low morbidity. BAL provides a method of examining the end-organ rather than an intermediary carrier of immunocompetent cells from the bone marrow. It has, however, limited value in the diagnosis of lung diseases.

Eosinophilic pneumonia is a disease characterized by lung infiltrates and peripheral eosinophilia. It is caused by a variety of agents including drugs, fungi, parasites and inhalants. Previous reports concerning BAL findings in eosinophilic pneumonia have shown that eosinophils (and sometimes lymphocytes) are increased in bronchoalveolar lavage fluids (BALF). This observation is essentially the same as that in allergic bronchial asthma, which is often accompanied by eosinophilic pneumonia and seems to have a similar pathogenic mechanism.

We describe a case of eosinophilic pneumonia with asthma in which BALF contained a considerable number of mature plasma cells and abundant lymphocytes. These observations may have important implications in the local maturation of B cell lineage and local production of immunoglobulins in eosinophilic pneumonia.

Case Report

A 49-year-old nonsmoking man was admitted to Hokkaido University Hospital in October, 1986 for the evaluation of wheezing and abnormal lung shadows. He first experienced episodes of wheezing ten years previously and had been treated intermittently with bronchodilators and corticosteroids at another hospital. The attacks of wheezing had been aggravated gradually over the past six years. In September, 1986, he presented to the hospital complaining of a low-grade fever and was found to have roentgenographic abnormalities. He was treated with various antibiotics without benefit. One month later, he was referred to our hospital for further investigation.

Although his past history was unremarkable, his grandmother had had asthma. He had been working at a feed factory for the past 24 years. He stated that he had been inhaling a large amount of dust consisting of corn, wheat, bean refuse, and bran. Chest auscultation revealed inspiratory rhonchi over the middle and lower lung bilaterally and inspiratory fine crackles over the right lung. The remainder of the physical examination was unremarkable. A chest

![FIGURE 1. Posteroanterior roentgenogram on admission shows bilateral infiltrates in the lung periphery. The general pattern is alveolar, as indicated by the presence of possible air bronchograms.](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21571/ on 06/26/2017)
The chromatin (mg/dl) inal dominate in IgE/albumin these morphologic a indicates eosinophils. Vacuoles which obscure the nucleus. Vacuoles having a halo near one side of the nucleus. Vacuoles are present near the cell border. Arrowheads indicate eosinophils having a bilobed nucleus and eosinophilic granules (May-Grünewald-Giemsa staining). B (lower). Arrow again indicates a plasma cell. Its nucleus is ovoid and eccentrically located. The chromatin is extremely coarse and arranged in the nucleus like the numbers on a clock. The cell border is irregular and flabby. All these morphologic features are characteristic of plasma cells. Arrowhead indicates a mast cell whose cytoplasm is packed with basophilic granules, which obscure the nucleus. Note that lymphocytes predominate in the preparation (May-Grünewald-Giemsa staining, original magnification × 330).

x-ray film on admission (Fig 1) showed patchy infiltrates in the right middle to lower and left middle lung fields.

Routine laboratory data revealed a hemoglobin of 12.6 g/dl, a leukocyte count of 6,800/cu mm, with 54 percent polymorphonuclear cells, 22 percent lymphocytes, 5 percent monocytes, 1 percent basophils, 18 percent eosinophils, and a platelet count of 36.9×10⁹/cu mm. The absolute number of eosinophils was 1,788 cu mm. Serum immunoglobulins were: IgG, 1,510 mg/dl; IgA, 179 mg/dl; IgM, 112 mg/dl. IgE was elevated dramatically at 7,070 IU/ml (normal <400 IU/ml). Pulmonary function measurements included VC 5.59 L (149 percent of predicted); FEV₁/FVC 74 percent; FRC 4.6 L (142 percent); TLC 7.7 L (134 percent); RV 2.1 L (118 percent); Dco, 24.6 ml/min/mm Hg (111 percent).

BAL was performed with a bronchofiberscope wedged into the lateral rami of the right upper anterior segment where lung infiltrates were present. A total of 180 ml of sterile saline solution was instilled with a recovery rate of 26 percent. Cytocentrifuge slides were made and stained using the May-Grünwald-Giemsa technique. Differential cell analysis revealed 56.6 percent lymphocytes, 26.3 percent eosinophils, 14.4 percent macrophages, 1.3 percent mast cells, 1.3 percent plasma cells (Fig 2), and 0.1 percent neutrophils, with a markedly increased total cell count of 2.9×10⁶ cells/ml of BALF. (The normal BAL differential for nonsmokers in our laboratory, as mean ± SEM, is: alveolar macrophages 89.1±1.5 percent, lymphocytes 10.6±1.5 percent, neutrophils 0.4±0.1 percent, eosinophils 0±0 percent. The normal total cell count is 10.2±1.8×10⁶ cells per ml of BALF.) The T cell subpopulations were: OKT-3, 93.3 percent; OKT-4, 57.9 percent; OKT-8, 32.8 percent. The OKT-4/8 ratio was 1.8.

BALF was concentrated 50-fold by a dialysis tube at 4°C. The albumin level in the concentrated BALF was determined by single radial immunodiffusion method using commercially available agar plates (LC-Partigen-Albumin, Hoechst Japan, Tokyo). IgE was measured by radioimmunosolvent method. Two nonsmoking patients with lung cancer and one nonsmoking healthy volunteer (mean age, 45±22 yr; range, 20 to 58 years; BALF macrophages, 89.0±5.6 percent; lymphocytes, 10.8±5.7 percent; neutrophils, 0.2±0.2 percent, eosinophils, 0.1±0.1 percent) served as control subjects.

As shown in Table 1, the IgE level and the ratio of IgE to albumin in BALF from the present case were markedly elevated as compared with those in the control subjects. Moreover, the ratio of IgE to albumin in BALF from our patient was higher than that in serum, indicating local production of IgE.

Transbronchial lung biopsy was performed at right basal segments and microscopic examination showed mild infiltration of lymphoid cells, eosinophils, and plasma cells in the alveolar septa and bronchial walls consistent with the diagnosis of eosinophilic pneumonia. Granulomas and vasculitis were not found.

Although allergic bronchopulmonary aspergillosis was contemplated, serum screening by the double diffusion method for precipitins against ten antigens (Hollister-Stier, Spokane, WA), including Aspergillus fumigatus, produced negative results. Skin testing for immediate cutaneous reactivity was negative with all 24 antigens (Torii Pharmaceutical Co, Tokyo), including A. fumigatus.

Since the grain dusts he had inhaled must have contained a large number of contaminants (including seeds, pollen, bacteria and their endotoxins, fungi and their metabolites, insects, quartz, and chemical pesticides and herbicides,) it was highly possible that he had been sensitized to some of these materials. Therefore, he was diagnosed as having eosinophilic pneumonia of undetermined origin with asthma.

The patient was started on therapy with 40 mg of oral methylprednisolone daily and experienced marked improvement in his symptoms and roentgenologic abnormalities after three days. The doses of the drug were tapered and he has been kept on a maintenance dose (methylprednisolone 4 mg daily) without recurrence.

### Table 1—IgE and Albumin Levels in Concentrated Bronchoalveolar Lavage Fluids (BALF) and Serum

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<th>Control subjects (BALF)</th>
<th>Present case</th>
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<td></td>
<td>Lung cancer</td>
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<td>IgE (IU/ml)</td>
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<td>albumin (mg/dl)</td>
<td>177</td>
<td>166</td>
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<td>IgE/albumin ratio (IU/mg)</td>
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Although the mechanism by which inflammatory cells accumulate at the site of disease has not been fully elucidated, there are two major possibilities: recruitment from peripheral blood or local proliferation. Local differentiation may follow these processes, as exemplified by the fact that monocytes mature to be resident alveolar macrophages.

Our patient had a considerably elevated proportion of plasma cells in BALF. So far as we know, this is the first case of eosinophilic pneumonia in which plasma cells appeared in BALF. It is unlikely that these plasma cells were attracted from blood and migrated into the alveolar spaces since plasma cells were not found in his blood. Therefore, the presence of plasma cells in BALF suggests local differentiation of B cells into immunoglobulin-producing plasma cells.

In interstitial lung diseases such as hypersensitivity pneumonitis, in which antibody formation is involved in the major pathogenic mechanism, a large proportion of antibodies is thought to be produced locally at the site of disease. Functional proof of this mechanism is the fact that plaque-forming cells that secrete specific antibody have been found in BALF after intratracheal antigen challenge in mice. We have also found that specific plaque-forming cells to *Mucor polyspora faeni* (the most prevalent causative fungus in farmer's lung disease) appear in BALF from subcutaneously and intratracheally sensitized rabbits (unpublished observation). Our present case adds morphologic proof to these findings.

The fact that IgE/albumin ratio in BALF is higher than that in serum can be taken as evidence of local production of IgE. Immunoglobulins thus produced may increase, in turn, their serum concentrations. Although the classes of immunoglobulins produced by the plasma cells were not determined, they would certainly have contributed to the extremely high level of serum IgE as well as BALF in the patient.

The presence of plasma cells does not seem to be a phenomenon specific to eosinophilic pneumonia. It has been reported that immunoblasts and/or plasma cells were found in BALF in immunocompromised patients with or without *Pneumocystis carinii* pneumonia. In general, chronically inflamed lesions contain a greater or lesser number of lymphoid or plasmacytoid cells on microscopic examination. Therefore, the presence or absence of plasma cells in BALF may simply reflect the density of the cells on alveolar walls and the degree of the filling of alveolar spaces by the cells.

Another notable observation in the present case is that BALF contained a markedly high number of lymphocytes. Most interstitial lung diseases have a more or less increased proportion of lymphocytes. Although the role of lung lymphocytes in the pathogenesis of lung diseases is not fully known, several reports have revealed that they are intimately involved in the mechanism of formation and maintenance of pulmonary lesions by releasing a variety of lymphokines. The process in which B cells proliferate and differentiate has recently been investigated extensively and a deeper insight into the lymphokine network has been obtained. It is generally accepted that T cell-derived cytokines, such as B cell growth factor (BCGF) and B cell differentiation factor (BCDF), play a major role in B cell stimulation. It is also now clear that interleukin-2 and interferon-y which are well-known as T cell tropic factors, can work directly on B cells. Evidence has also been presented that macrophage-derived cytokine, interleukin-1, is involved in B cell activation. Since most BALF lymphocytes in our case were T cells (as determined by OKT-3 antibody,) it is highly possible that the lung lymphocytes were releasing these factors other than the known lymphokine, such as eosinophil chemotactic factor (ECF), thereby contributing to the differentiation of B cells and production of antibodies. However, direct proof of this possibility remains to be presented.

In summary, the present case is important in two respects. First, the appearance of plasma cells in BALF and the relative increase of IgE/albumin ratio in BALF compared to serum mean that immunoglobulins are produced at the site of disease, and suggests the presence of lymphokine(s) responsible for the differentiation of B cells into plasma cells. Second, the increased proportion of BALF lymphocytes suggests their roles in the local differentiation of B cells.

The local immune processes in the lung, like those in other organs, are likely to be regulated by dynamic and intricate cellular interactions. These interactions are thought to be mediated by a variety of cytokines, mediators, and physical contacts made by immunocompetent cells. The wide variety of cell types in BALF from our patient possibly reflects such complicated immune processes in eosinophilic pneumonia and suggests the existence of a cytokine network.

References

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