ORIGINAL ARTICLE

Extrinsic allergic alveolitis: comparative study of the bronchoalveolar lavage profiles and radiological presentation

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Introduction: Extrinsic allergic alveolitis (EAA) is an immunologically mediated interstitial lung disease. The abnormalities in the bronchoalveolar lavage (BAL) fluid cell counts are almost always seen in patients with EAA according to the stage of the disease. The aim of this retrospective study was to find out how the BAL lymphocyte count, percentage of lymphocytes expressing HLA-DR, CD4/CD8 T cell ratio in BAL fluid, and the concentration of immunoglobulin G in serum correspond to the inflammatory activity of the disease.

Methods: The study included 14 patients with EAA. BAL fluid samples were obtained and processed for cytological and cytometric analysis. Immunoglobulin G serum concentrations were measured. High resolution computed tomography (HRCT) scoring system modified by Gay was used for establishing the alveolar and interstitial score in each patient.

Conclusions: It was found that subjects with normal value of CD4/CD8 ratio in BAL fluid had higher interstitial HRCT score. Clinical presentation, continuous exposure to the causative antigens, and BAL lymphocyte count positively correlated with the alveolar HRCT score. It is proposed that the increased BAL lymphocyte count could be the predictor of the inflammatory activity of the disease, especially in people with lasting exposure to the offending antigen.

Extrinsic allergic alveolitis (EAA) belongs to the family of interstitial lung diseases. It results from repeated inhalation of causative antigens in susceptible people. The prevalence of the disease is unknown. It is probable that both humoral and cellular mechanisms participate in the development of the lung lesion after the repeated inhalation of offending antigens. For the acute form of the disease, which occurs several hours after the antigen challenge, an immune complex mediated tissue injury is typical.^{1,2} On the other hand, T cell mediated immune inflammatory response prevails in advanced stages of the disease.³

The clinical presentation of the disease has been defined as acute, subacute, and chronic. According to recent knowledge, the disease could be retrospectively classified as acute intermittent, acute progressive, chronic progressive, and chronic non-progressive.⁴

Exposure to known sensitising antigens is the most important diagnostic sign.⁴ The typical high resolution computed tomography (HRCT) findings, bronchoalveolar lavage (BAL), and in some cases the surgical lung biopsy are also important for diagnosis of EAA.⁵

The aim of our study was to investigate how BAL lymphocyte count, lymphocytes expressing HLA-DR, CD4/CD8 T cell ratio in BAL fluid, and serum concentration of immunoglobulin G (IgG) correspond to the inflammatory activity of the disease represented by HRCT alveolar score and to the chronicity of the disease represented by HRCT interstitial score.

METHODS

Subjects (table 1)

Our study was retrospective. Fourteen patients with EAA diagnosed at our clinic between 1999 and 2004 were investigated. There were eight women and six men, with the mean (SD) age of 59 (13) years. Seven patients presented with the subacute form of EAA, another seven presented with

the chronic form of the disease. All of them underwent bronchoscopy including BAL, the cytological and cytometric examination of BAL fluid, and HRCT of the chest. Serum concentrations of IgG were measured. None of the patients were treated with corticosteroids or immunosuppressive therapy at the time of the diagnosis. We divided the patients into three groups according to the clinical presentation: acute, subacute, and chronic form of the disease. The exposure to a known offending antigen at the time of the diagnosis was important.

Bronchoalveolar lavage, cytological, and cytometric analysis of BAL fluid (table 2)

BAL was performed with a flexible bronchoscope (Olympus, Olympus Optical, Japan) wedged into segmental or subsegmental middle lobe bronchus. Four 50 ml aliquots of sterile 0.9% saline solution were instilled and then aspirated. The first three doses were processed for differential cell counts, with the use of May-Grünwald and Giemsa-Romanovski stains. The fourth dose of BAL underwent cytometric investigation. Five differently immunofluorescent labelled leucocyte suspensions were prepared. The data were collected in the flow cytometer FACS Calibur, and analysed with the software CellQuest and Claris. CD 3+ and CD 14 + cell subsets were separated. Subpopulations of CD 4+, CD 8+, and HLA-DR + T cells were expressed as percentage of the entire CD 3+ population.

Serum IgG (table 2)

The serum concentrations of IgG were estimated. The reference range was 6.9–14.0 mg/l.

Abbreviations: EAA, extrinsic allergic alveolitis; BAL, bronchoalveolar lavage; HRCT, high resolution computed tomography; IgG, immunoglobulin G

Table 1 Clinical and exposure data of the study group

Patient number	Age	Sex	Clinical presentation	Exposure*	Antigen†	Histology‡
1	69	F	chronic	—	OD	—
2	52	F	subacute	+	OD	+
3	71	F	chronic	—	OD	+
4	55	M	subacute	+	H	+
5	59	M	subacute	+	B	—
6	75	F	chronic	+	B	—
7	50	M	chronic	—	MF	—
8	76	F	chronic	—	H	—
9	76	F	chronic	+	H	—
10	46	M	subacute	+	MF	+
11	52	M	chronic	—	B	—
12	51	F	subacute	+	MF	+
13	32	M	subacute	—	OD	+
14	57	F	subacute	—	B	+

*Exposure, + exposure to the causative antigen continuing at the time of the diagnosis, — exposure to the causative antigen discontinuing at the time of the diagnosis. †Antigen—OD, organic dusts except of hay; H, hay; B, birds (parrots, parakeets, etc); MF, metalworking fluid. Histology, + surgical lung biopsy was performed, — surgical lung biopsy was not performed.

HRCT scoring system (table 3)

HRCT scans were performed using the Somatom Sensation Four (Siemens, Germany) scanner. They were scored by an experienced radiologist, who used the modified scoring system by Gay.⁷ The radiologist scored alveolar and interstitial scores at four levels of the right and of the left lung. These scores were summed for each patient and the average interstitial and alveolar scores were calculated.

Statistical analysis

Statistical software BMDP-PC 90 was used for statistical analysis and the Mann-Whitney test applied. A p level <0.05 was regarded as significant. We did not count the significance levels for the data characterising the subgroups of the patients, as the number of patients in each subgroup was too small. The number of investigated patients was limiting for the statistical analysis.

RESULTS

It is apparent that the patients with the subacute form of EAA had higher alveolar scores than those suffering from the chronic form of the disease (tables 4 and 5).

We also found the correlation of the clinical presentation and the alveolar score ($p = 0.02$). The clinical presentation of the disease had no significant correlation with the interstitial score ($p = 0.18$). The patients who were exposed to the inhalation antigen at the time of the diagnosis had higher alveolar score than the patients who were not exposed

($p < 0.01$). The exposure to the antigen at the time of the diagnosis had no significant correlation with the interstitial score ($p = 0.18$). Concentrations of the serum IgG correlated neither with the alveolar ($p = 0.26$) nor with the interstitial score ($p = 0.68$). Higher alveolar score in the patients with higher BAL lymphocyte count reached significance compared with the normal BAL lymphocyte count group ($p = 0.05$). We did not find any correlation of BAL lymphocyte count and the interstitial score ($p = 0.68$). We did not find any correlation of the CD4/CD 8 T cell ratio in BAL fluid and the alveolar score ($p = 0.24$). The patients with normal CD4/CD8 T cell ratio had significantly higher interstitial score ($p = 0.01$). The correlation of the percentage of HLA-DR+ T cells in BAL fluid and the alveolar ($p = 0.13$)/interstitial score ($p = 0.41$) was not found.

We have noticed the significance of the sex and age; women in our group had higher interstitial scores ($p = 0.02$). The people with the chronic EAA were significantly older than the patients with the subacute form of EAA ($p = 0.04$).

Higher concentrations of IgG were seen in the serum of the patients with subacute EAA and in the serum of the subjects exposed to the offending antigen (table 5). Normal serum concentrations of IgG prevailed in the group of the patients with the chronic form of EAA and in the people not exposed to the antigen at the time of the diagnosis. Higher BAL lymphocyte count was found in the subjects exposed to the offending antigen at the time of the diagnosis. Prevailing BAL lymphocyte count was less obvious among the patients with

Table 2 Cytological and cytometric analysis of BAL fluid and serum IgG concentrations

Patient number	L (%)	PMN (%)	AM (%)	E (%)	CD4/CD8	HLA-DR (%)	IgG (mg/l)
1	10	16	72	2	3.17	7	13.24
2	80	9	8	3	0.19	86	23.89
3	48	18	24	8	0.93	90	12.02
4	6	78	16	0	0.22	91	15.65
5	59	22	26	3	0.69	55	8.79
6	22	18	58	2	3	16	5.25*
7	8	4	88	0	0.5	4	10.86
8	12	6	89	0	1.34	72	15.88
9	48	5	43	4	0.39	23	13.51
10	51	8	78	0	0.22	41	17.75
11	14	8	72	0	0.23	23	12.47
12	20	7	77	3	0.24	63	15.91
13	12	2	41	4	1.19	43	9.71
14	53	8	41	0	0.79	45	17.26

L, BAL lymphocyte count (reference range to 15%); PMN, BAL polymorphonuclear neutrophil count (reference range to 3% in non-smokers, to 5% in smokers); AM, BAL alveolar macrophage count; E, BAL eosinophil count (reference range to 0.5%); CD4/CD8 T cell ratio in BAL fluid (reference range 0.90–3.60); HLA-DR + lymphocytes in BAL fluid (reference range 4%–17%). *A patient suffering from dysgammaglobulinaemia IV.

Table 3 Evaluation of HRCT scans, the alveolar and interstitial scoring system

Alveolar score	Interstitial score
0 No alveolar disease	0 No interstitial disease
1 GGO involving <5% of the lung parenchyma	1 Interlobular septa thickening, no discrete honeycombing
2 GGO involving up to 25% of the lung parenchyma	2 Honeycombing involving up to 25% of the lung parenchyma
3 GGO involving 25%–49% of the lung parenchyma	3 Honeycombing involving 25%–49% of the lung parenchyma
4 GGO involving 50%–75% of the lung parenchyma	4 Honeycombing involving 50%–75% of the lung parenchyma
5 GGO involving >75% of the lung parenchyma	5 Honeycombing involving >75% of the lung parenchyma

GGO, glass ground opacity. Alveolar score, inflammatory activity of the disease. Interstitial score, rate of pulmonary fibrosis, predominantly seen in the chronic forms of EAA.

the chronic form of the disease. Lower CD4/CD8 T cell ratio was seen in the patients with the subacute form of EAA and in the patients with continuous exposure to the offending antigen. Fifty seven per cent of the patients suffering from the chronic EAA had a normal CD4/CD8 T cell ratio.

DISCUSSION

Some authors suggest that the serum IgG concentrations are associated with the severity of the disease.⁸ Higher serum IgG concentrations were found in the patients with the subacute form of the disease and in the patients exposed to the sensitising antigen. We did not find any significant correlation of the alveolar/interstitial score and serum IgG concentrations.

Thirty per cent of lymphocytes in BAL fluid in non-smokers and 20% in smokers are usually taken as a diagnostic sign in literature, some authors suggest 40% as the arbitrary level.^{9–10} We would like to point out that we have not found any study concerning BAL lymphocyte count in patients with the acute, subacute, and chronic form of EAA. We have not found any study dealing with BAL lymphocyte count in patients with/without continuous exposure to the known offending antigen. According to our experience and to the studies of other authors, BAL lymphocyte count is not always increased in the patients with chronic EAA.^{11–12} Welker claims that BAL cell count in itself provides substantial diagnostic information only in such diseases like sarcoidosis and usual interstitial pneumonia, and are less helpful in EAA.^{13–14} The BAL lymphocyte count was higher in the patients with subacute EAA as well as in the subjects exposed to the inhalation antigen at the time of the diagnosis. Those with increased BAL lymphocyte count had higher alveolar HRCT scores ($p = 0.05$). It was previously found that there is no

correlation between the number of lymphocytes in BAL fluid and the outcome of the disease.¹⁵ It is proposed that the increased BAL lymphocyte count could be the predictor of the inflammatory activity of the disease.

Previous studies have shown that the CD4/CD8 ratio in BAL fluid may vary significantly according to the clinical presentation of the disease, the biological characteristics of the inhalation antigen, the exposure to the causative antigen at the time of the diagnosis, the immune susceptibility of particular patients, and to the history of smoking.¹⁶ Our data support the hypothesis of the correlation of the continuous exposure to the inhalation antigen and the low CD4/CD8 ratio in BAL fluid. Murayama raises the possibility that increased CD8 + T cells might have a protective effect against pulmonary fibrosis while comparatively increased CD4+ T cells might play an important part in the pathogenesis of pulmonary fibrosis in EAA.¹⁷ We proved that the persons with the normal CD4/CD 8 ratio had higher interstitial scores.

It was reported in previous studies and also noted in our study group that the patients with exposure to the causative antigen at the time of the diagnosis have increased count of HLA-DR+ T cells in BAL fluid.^{18–19}

Previous studies suggest that there was increased percentage of alveolar macrophages in the BAL fluid of the subjects with acute form of EAA. Our patients with subacute and chronic form of the disease did not had increased alveolar macrophage count in BAL fluid.²⁰

We found only a few studies using a scoring system for evaluation of HRCT scans. We found HRCT scoring system useful for evaluation of the inflammatory activity of the disease.

The average age of the subjects in our study group was 58.6 years. We found the patients suffering from the chronic form

Table 4 Alveolar and interstitial HRCT scores of the study group

Patient	Clinical presentation	Alveolar	Interstitial
1	chronic	1	4
2	subacute	4	1
3	chronic	3	2
4	subacute	5	1
5	subacute	3.5	1
6	chronic	3	3
7	chronic	0	1
8	chronic	0	4
9	chronic	3	3
10	subacute	3	1
11	chronic	1	1
12	subacute	5	2
13	subacute	3	3
14	subacute	2	3

Table 5 Subjects' characteristics before the clinical presentation of the disease and to the exposure to the causative antigen at the time of the diagnosis

	Subacute	Chronic	Exposure+	Exposure –
Increased IgG	71%	14%	57%	29%
Normal IgG	29%	86%	43%	71%
Increased BAL lymphocyte count	71%	43%	86%	29%
Normal BAL lymphocyte count	29%	57%	14%	71%
Decreased CD4/CD8	86%	43%	86%	43%
Normal CD4/CD8	14%	57%	14%	57%
Increased HLA-DR +	100%	57%	86%	71%
Normal HLA-DR +	0%	43%	14%	29%
HRCT alveolar	2.9	1.6	3.5	1.4
HRCT interstitial	1.7	2.6	1.7	2.6

of EAA older than the subacute group. This finding supports the hypothesis that the interstitial lung diseases tend to have a chronic course in older patients.

Our study was limited by the number of the patients studied. There were no patients with the acute form of EAA. This is probably caused by the fact that the acute form of EAA is often unrecognised or misdiagnosed because of the short duration of its signs and symptoms.

CONCLUSIONS

We showed that the clinical presentation, the lasting exposure to the causative antigen and the BAL lymphocyte count positively correlated with the inflammatory activity of the disease, reported by the alveolar HRCT score. Additionally we found that the patients with a normal CD4/CD8 ratio in BAL fluid had higher interstitial HRCT scores. It is proposed that the increased BAL lymphocyte count may be the predictor of the inflammatory activity of the disease, especially in persons with lasting exposure to the offending antigen.

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