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Bronchoalveolar lavage in extrinsic allergic alveolitis: effect of time elapsed since antigen exposure

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ABSTRACT: The aim of the present study was to investigate whether bronchoalveolar lavage (BAL) cell profile and immunoglobulin levels from patients with extrinsic allergic alveolitis (EAA) were related to the time elapsed between last antigen exposure and BAL.

For this purpose, an analysis was performed of BAL fluid (BALF) obtained from 59 nonsmoking EAA patients at various time-points after termination of antigen exposure and BAL.

BALF early after antigen provocation (group 1: <24 h) contained high absolute and relative numbers of lymphocytes, neutrophils, eosinophils and mast cells, and a low relative number of alveolar macrophages. When obtained after recent antigen exposure (group 2: 2–7 days), BALF showed high numbers of lymphocytes, plasma cells and mast cells, and high levels of immunoglobulins M, G and A (IgM, IgG and IgA). In BAL obtained one week or more after the final antigen exposure, (Group 3: 8–30 days; Group 4: 1–12 months) the distribution of all constituents showed a tendency to return to normal values, with the exception of the lymphocytes.

These results demonstrate that BAL cell profile and immunoglobulin levels in EAA are highly dependent on the time-point at which the material is obtained in relation to the last exposure to the causative antigen.

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Extrinsic allergic alveolitis (EAA), or hypersensitivity pneumonitis, is a disease initiated by repeated exposure to extrinsic organic antigens in susceptible indi-viduals [1, 2]. The clinical manifestation of EAA shows considerable variation as it is related to the frequency and intensity of exposure to the causative agent [3, 4].

The initial phase of EAA is characterized by an early increase in bronchoalveolar lavage fluid (BALF) polymorphonuclear neutrophils (PMNs) [1, 2, 5, 6]. Twelve hours to several days following onset, the bronchioloalveolitis consists mainly of CD8+ T-cells [7], which among others, modulates the B-cell response and antibody production by plasma cells [1, 7, 8]. Increase in the CD8+ T-cells in BALF results in a relatively low CD4+/CD8+ ratio [1, 2, 7]. After weeks to months following antigen exposure, a slight predominance of CD4+ T-cells is found in BALF [1, 7, 9].

Various immune mechanisms are involved in the pathogenesis of EAA [1]. Among these, immunoglobulins (Igs) [10–12], immune complexes [13, 14], complement, cytokines [15], lipids and other biological modifiers have been described as playing an important role [1]. In addition, T-cells have been implicated in the mechanism underlying tissue damage and repair in EAA [1].

As the relative number of lymphocytes and the balance of the various T-cell subsets present in BALF may be of predictive value with regard to nature (and outcome) of the pathological process [16–20], the timing of the BAL seems to be critical for the detection of characteristic constituents.

The aim of this study was to establish the relationship between the timing of BAL and the last exposure to the causative antigen on BAL-profiles of T-cells, other cells and proteins in EAA patients and to detect specific features of the various categories.

Patients and controls

Bronchoalveolar lavage fluid samples, obtained from patients suffering from EAA (n=67) during a 10 yr period between 1980 and 1990, were studied. The EAA patients presented with generalized constitutional and pulmonary symptoms, *i.e.* cough, dyspnoea and sometimes fever and chills. Although the symptoms were mostly transient, initial complaints and exacerbations occurred with repeated exposure or provocation to the causative antigen. The major physical findings were tachypnoea, cyanosis, and bilateral crepitant rales. Both

Table 1. – Characteristics of the groups studied: nonsmoking extrinsic allergic alveolitis (EAA) patients and controls

Groups	n	Age* yrs	F/M	Precipitins
EAA patient categories				
1) <24 h	14	53 (23–75)	4/10	$7^{A};3^{C};4^{D\#}$
2) 2–7 days	28	51 (27–70)	7/21	$18^{A};5^{B};2^{C};3^{D\#}$
3) 8–30 days	11	45 (19–71)	4/7	$8^{A};3^{C\#}$
4) 1–12 months	6	55 (25–73)	2/4	$4^{A};1^{B};1^{C\#}$
Controls	28	39 (19–60)	12/16	Not done

*: data indicate mean with range in parentheses; n: number of cases; F/M: female/male; #: avian proteins, all birds; A: pigeon; B: budgerigar; C: canary; D: parrot.

restrictive and obstructive defects in pulmonary function were observed. In most cases, the carbon monoxide diffusion capacity, measured when the patient was admitted to the hospital, was seriously disturbed. In some patients, hypoxaemia showed a substantial worsening with exercise. The diagnosis of EAA was based with care on clinical information, chest X-ray film, the presence of precipitating serum antibodies against the suspected antigens, pulmonary function tests and disappearance of symptoms after avoidance of antigen exposure. An open lung biopsy was performed in six patients, without sufficient clinical criteria to make a definitive diagnosis, and the diagnosis of EAA was verified histologically.

Fifty nine nonsmoking EAA patients were included, all of whom were frequently exposed to birds, *i.e.* pigeons, parrots, budgerigars or canaries (table 1). No patient was receiving corticosteroid treatment before BAL.

At the time of the lavage, these patients were divided into four categories, based on the time period between the presumed termination of antigen exposure and the BAL: group 1: < 24 h; group 2: 2–7 days; group 3: 8–30 days; and group 4: 1–12 months. Antigen provocation was achieved by bringing the patient into contact with the birds, followed by a BAL within 24 h.

The control group consisted of 28 nonsmoking healthy volunteers, without chest abnormalities or a history of pulmonary abnormalities or disease, and without contact to EAA inducing antigens (table 1).

Methods

Bronchoalveolar lavage

BAL was performed, as reported previously, during fibreoptic bronchoscopy [2]. The procedure is briefly described. After premedication (atropine and sometimes diazepam or codeine), and local anaesthesia of the larynx and bronchial tree (tetracaine 0.5%), BAL was performed by standardized washing of the right middle lobe with four aliquots of 50 ml sterile saline (0.9% NaCl) at room temperature.

Recovered BALF was kept on ice in a siliconized specimen trap, and was separated from cellular compounds by centrifugation (for 5 min with a force of 350×g). Supernatants were directly stored at -70°C after an additional centrifugation step (for 10 min with a force of 1,000×g). The cells were washed twice, counted and suspended in minimal essential medium (MEM; Gibco, Grand Island, New York, USA) supplemented with 1% bovine serum albumin (BSA; Organon, Teknika, Boxtel, The Netherlands).

Preparations of the cell suspensions were made in a cytocentrifuge (Shandon). Cytospin slides of BAL cells were stained with May-Grünwald-Giemsa (MGG; Merck, Darmstadt, Germany) for cell differentiation. At least 1,000 cells were counted.

Simultaneously with the BAL, peripheral blood samples were taken. Mononuclear cells were isolated from heparinized blood by Ficoll-isopaque density centrifugation (Pharmacia, Uppsala, Sweden).

If more than 15% lymphocytes were present in BALF, T-cell (sub)populations in BALF and also in blood were determined. Total T-cells and subpopulations were recognized by staining with monoclonal antibodies CD2 (OKT11), CD3(OKT3), CD4(OKT4) and CD8(OKT8) (Orthopharmaceuticals, Diagnostic systems, Beerse, Belgium). Identification of T-cells reacting with monoclonal antibodies was performed by means of a conventional indirect immunofluorescence technique using fluorescein isothiocyanate (FITC)-labelled goat-anti-mouse-Ig (GAM, Nordic, Immunological Laboratories, Tilburg, The Netherlands, and from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam, The Netherlands).

For the quantitative determination of albumin in serum and BALF the albumin method was used. The albumin method is an adaption of the bromocresol purple (BCP) dye-binding method [21, 22]. In short, this method is as follows. In the presence of a solubilizing agent, BCP binds to albumin at pH 4.9. The amount of albumin-BCP complex is directly proportional to the albumin concentration. The complex absorbs at 600 nm. Albumin concentrations in serum and BALF were expressed in $g \cdot l^{-1}$ and $mg \cdot l^{-1}$, respectively.

IgM, IgG and IgA concentrations in BALF were determined by an enzyme-linked immunosorbent assay (ELISA) method; microtitre plates were coated with a rabbit anti-human-isotype antiserum (anti-IgM, (CLB, Amsterdam, The Netherlands), anti-IgG and anti-IgA (Dako, Glostrup, Denmark)). Bound Igs from BALF were visualized by using a horseradish peroxidase (HRP)-labelled rabbit anti-human-Ig antiserum (with anti-IgA, -IgG, -IgM, -kappa, -lambda reactivity (Dako, Glostrup, Denmark)) and a chromogenic substrate orthophenyl diamine (OPO; (Baker, Chemicals BV, Deventer, The Netherlands). Concentrations in BALF were expressed in $mg \cdot l^{-1}$ using as a reference a commercial human standard serum, HOO-03 (CLB, Amsterdam, The Netherlands).

Statistical analysis

Data are expressed as mean±standard error of the mean (SEM). In order to investigate whether there were statistically significant differences between the four categories of EAA patients data were analysed by the Kruskal-Wallis one-way analysis of variance (ANOVA) test. Each category denoted a certain moment

of observation after the last antigen exposure. The Mann-Whitney test was subsequently used to evaluate the differences between each category and the control group. A p-value of <0.05 was considered to be significant.

Results

The mean numbers of cells (±SEM) in BALF samples obtained from EAA patients and control subjects, as well as the CD4+/CD8+ ratio and protein levels in BALF and peripheral blood are summarized in tables 2–4 and figures 1 and 2.

Category 1: < 24 h

Category 1 (BAL after antigen provocation) was characterized by the highest absolute and relative numbers of polymorphonuclear neutrophils (PMNs), as well as the highest percentage of eosinophils, and a low percentage of alveolar macrophages (AMs). As compared with the control group, the total cell count, lymphocytes, plasma cells, and mast cells were also increased (fig. 1 and table 2). The percentage of T-cells (CD3+) and of CD8+ T-cells were also significantly increased compared with the controls (table 3). Of all groups studied, the percentage of CD4+ T-cells was highest in group 1, whereas the percentage of CD8+ T-cells was relatively low in this group (table 3). The CD4+/CD8+ ratio in BALF was low compared with the control subjects (fig. 2).

In BALF, the levels of albumin, Igs and Ig ratios to albumin were increased in category 1, and in the other categories (2, 3 and 4), as compared with the control subjects (table 4).

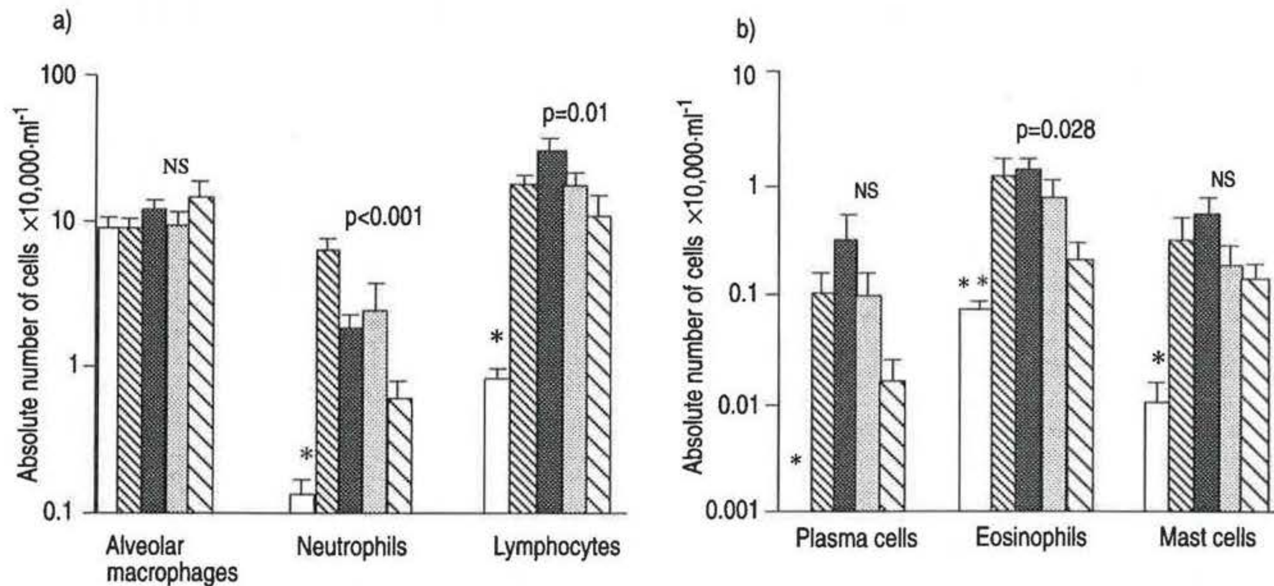

Fig 1. – The effect of time elapsed between bronchoalveolar lavage (BAL) and termination of antigen exposure on the absolute numbers of cell types in BALF of extrinsic allergic alveolitis (EAA) patients and control subjects (the Y-axis has a logarithmic scale). a) alveolar macrophages, neutrophils and lymphocytes; b) plasma cells, (value controls =0), eosinophils and mast cells. p: Kruskal-Wallis p-value between the four categories of EAA patients; NS: nonsignificant; *: p<0.05 Mann-Whitney U-test all EAA patient groups *vs* controls; **: p<0.05 Mann-Whitney U-test EAA patients belonging to category 1, 2 and 3 *vs* controls. ▭: controls; ▨: EAA<24 h; ■: EAA 2–7 days; ▒: EAA 8–30 days; ▧: EAA 1–12 months.

Table 2. – Cellular components in bronchoalveolar lavage fluid (BALF) of extrinsic allergic alveolitis (EAA) patients and controls: differential cell counts

Groups	TCC ×10⁴·ml⁻¹	Percentage of TCC AM	PMN	Lym	PC	Eos	MC
EAA patient categories							
1) <24 h	33.6±4.0#	29.0±3.0#	18.1±4.5#	48.2±4.7#	0.34±0.16#	3.55±1.14#	0.85±0.20#
2) 2–7 days	46.4±4.8#	30.2±2.6#	4.2±0.7#	61.3±2.7#	0.57±0.16#	2.70±0.60#	1.01±0.14#
3) 8–30 days	29.0±8.7#	41.3±5.6#	4.2±2.2#	52.0±4.5#	0.21±0.09#	1.70±0.49#	0.60±0.20#
4) 1–12 months	25.5±4.1#	55.5±8.3#	2.1±0.6	41.2±8.6#	0.05±0.03#	0.60±0.30	0.48±0.19#
p-value*	0.03	0.005	<0.001	0.008	0.05	0.05	NS
Controls	10.3±1.5	89.8±0.7	1.3±0.2	8.4±0.7	0.0±0.0	0.44±0.10	0.09±0.03

TCC: total cell count; AM: alveolar macrophages; PMN: polymorph neutrophils; Lym: lymphocytes; PC: plasma cells; Eos: eosinophils; MC: mast cells. *: Kruskal-Wallis p-value between the four categories of EAA patients; NS: nonsignificant; #: p<0.05 Mann-Whitney U-test EAA patient groups *vs* controls.

Table 3. – T-cell subpopulations recovered from bronchoalveolar lavage fluid (BALF) and CD4+/CD8+ ratios in BALF and peripheral blood (PB) of extrinsic allergic alveolitis (EAA) patients and controls

		BALF				PB
Groups	n	CD3+ %	CD4+ %	CD8+ %	CD4+/CD8+	CD4+/CD8+
EAA patient categories						
1) 24 h	10	83.7±3.1#	49.6±6.3	36.9±5.4#	1.75±0.34	1.62±0.45
2) 2–7 days	22	85.6±1.7#	44.3±4.1	40.6±2.9#	1.32±0.20#	1.43±0.13#
3) 8–30 days	8	86.0±1.9#	46.1±8.7	42.6±8.2#	2.07±0.82	3.28±0.64
4) 1–12 months	5	88.0±1.2#	46.6±9.9	41.6±9.8#	2.28±1.03	1.99±0.37
p-value*		NS	NS	NS	NS	0.01
Controls	6	72.8±2.0	50.5±3.4	23.0±4.2	2.60±0.17	1.75±0.33

*: Kruskal-Wallis p-value between the four categories of EAA patients; NS: nonsignificant. #: p<0.05 Mann-Whitney U-test EAA

Table 4. – Protein levels in bronchoalveolar lavage fluid (BALF) of extrinsic allergic alveolitis (EAA) patients and controls

Groups	l-alb mg·l^{-1}	l-alb/s-alb ×100	IgM mg·l^{-1}	IgM/l-alb	IgG mg·l^{-1}	IgG/l-alb	IgA mg·l^{-1}	IgA/l-alb
EAA patient categories								
1) <24 h	187±32#	0.45±0.08#	10.3±2.8#	0.06±0.01#	242±39#	1.62±0.35#	41.6±11.7#	0.22±0.04#
2) 2–7 days	187±20#	0.47±0.05#	19.0±4.2#	0.12±0.03#	243±43#	1.89±0.41#	59.7±13.2#	0.57±0.20#
3) 8–30 days	187±53#	0.52±0.15#	15.0±7.8#	0.08±0.01#	150±60#	0.87±0.23#	35.3±10.2#	0.17±0.04#
4) 1–12 months	117±19#	0.25±0.41#	3.6±1.2#	0.03±0.01#	85±25#	0.70±0.29#	23.9±8.4#	0.23±0.09#
p-value*	NS	NS	NS (0.06)	0.01	0.05	NS (0.07)	NS (0.06)	NS
Controls	71±8	0.19±0.02	0.4±0.1	0.01±0.002	11±2	0.16±0.02	3.6±0.7	0.05±0.01

alb: albumin; l-alb: BALF alb; s-alb: serum alb; *: Kruskal-Wallis p-value between the four categories of EAA patients; NS: nonsignificant; #: p<0.05 Mann-Whitney U-test EAA patient groups *vs* controls.

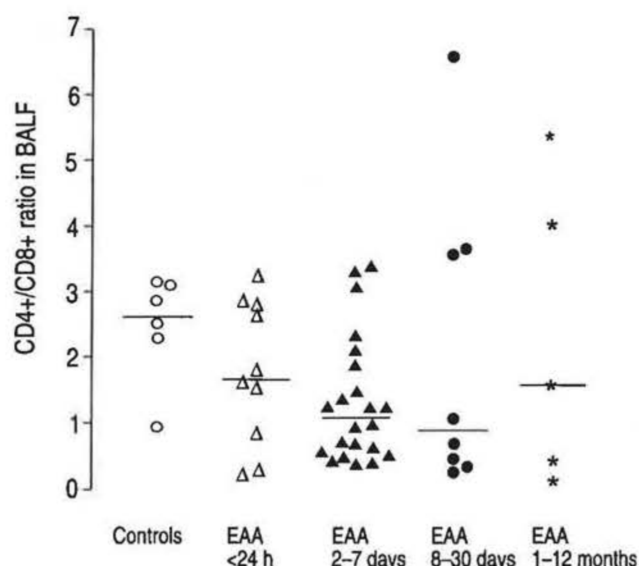

Fig. 2. – Individual CD4+/CD8+ ratios (with median values) in bronchoalveolar lavage fluid (BALF) obtained from the four categories of extrinsic allergic alveolitis (EAA) patients studied and control subjects.

Category 2: 2–7 days

Category 2 (BAL performed within 2–7 days after the last antigen exposure) showed, as the most characteristic feature, a drop in the percentage of PMNs compared with category 1 (table 2). Category 2 patients demonstrated the highest absolute and relative numbers of lymphocytes, plasma cells, eosinophils and mast cells, and the lowest CD4+/CD8+ ratio in BALF. The CD4+/CD8+ ratio was also low (1.43±0.13) in peripheral blood obtained from these patients. In addition, in BALF, the highest levels of the respective immunoglobulins IgM, IgG and IgA were found, and the IgM ratio to albumin in BALF samples obtained from patients of this category was the highest (table 4).

Category 3: 8–30 days

The median CD4+/CD8+ ratio in BALF was low compared with the control group (fig. 2). In contrast, the mean values showed no differences, because of bias due to the nonparametric distribution (table 3). Furthermore, no specific features were demonstrated in BALF samples obtained from patients with EAA which belong to this category in comparison with category 2, except for a lower total cell count (table 2).

Category 4: 1–12 months

Category 4 (BAL performed within 1–12 months after termination of the antigen exposure) had no specific characteristics, the numbers of eosinophils, plasma cells and PMNs in BALF were returning to normal, in contrast to the absolute and relative number of lymphocytes and mast cells, which appeared to be still increased. As a consequence, the percentage of AMs was also still decreased (fig. 1 and table 2).

Table 5. – Statistically significant differences of bronchoalveolar lavage fluid (BALF) constituents between four studied categories of extrinsic allergic alveolitis (EAA) patients

BALF constituents	<24 h	2–7 days	8–30 days	1–12 months
Alveolar macrophages %	↓			↑
Lymphocytes*		↑		↓
Plasma cells*		↑		↓
Neutrophils*	↑			↓
Eosinophils*	↑			↓
IgM-, IgG- and IgA-levels		↑		↓
Ratio IgM/albumin		↑		

*: Absolute and relative number; ↑: highest value; ↓: lowest value (for the exact values see tables 2–4 and fig. 1). IgM, G and A: immunoglobulin M, G and A.

Table 5 gives a summary of the most important significant differences between the four categories of EAA patients studied.

Discussion

In the present study, the cellular components and protein levels in BALF obtained from EAA patients were found to be related to the time elapsed between termination of antigen exposure and the actual performance of BAL for obtaining the material for analysis. The course of EAA may be divided into various phases according to subsequent immunological reactions in the lung [1, 20, 23]. Consequently, these phases of the immune response are reflected in a varying composition of BALF samples [1, 2, 20]. Patients in our study, lavaged within the first 24 h after antigen inhalation, showed the highest values of PMNs in BALF, in agreement with other studies [2, 5, 17]. When BAL was performed in the subsequent 2–7 days after the exposure to antigens, not only total cell count, but also the absolute and relative number of lymphocytes, plasma cells, eosinophils and Ig levels were found to be elevated. In view of this time-related effect on the composition of BALF in EAA, an analysis of a BALF sample may make it possible to determine whether antigen exposure has recently occurred.

The mechanisms underlying the rapid recruitment of inflammatory cells into the alveoli, finally resulting in an accumulation of lymphocytes [1, 2], are as yet not clear. It has been suggested that cell migration is triggered by antigen-antibody complexes and the release of cytokines by antigen specific T-cells in the course of the immune response [24, 25].

In contrast to the presumption that the presence of T-cells is predictive for the activity and progress of the disease [8], clinical symptoms were found to subside in longstanding EAA, whereas the number of T-lymphocytes in BALF remained increased [1, 5, 7]. Furthermore, in asymptomatic EAA patients and normal farmers the high numbers of T-lymphocytes in BALF can persist for 2 yrs or more [23, 26]. Thus, the increased numbers of T-cells, as such, in the BALF is not necessarily a marker for disease activity or progression [8], but subpopulations of T-cells may be of importance [26, 27]. Recently, TRENTIN *et al.* [17] showed a shift from the CD8+-predominant cellular profile in BALF toward the normal CD4+-predominant profile after termination of antigen exposure. Interestingly, in the present study, no such CD8+ predominance was observed in the BALF obtained immediately after challenge, but rather a higher percentage of CD4+ T-cells was demonstrated. However, when gathered during the subsequent 2–7 day time period, the mean CD4+/CD8+ ratio was lowest in both BALF and peripheral blood (PB), as compared with the other time intervals. Our data are not necessarily contradictory to those of TRENTIN *et al.* [17], because the latter authors did not perform a BAL within 1 week after antigen provocation. In our present study, an increase of CD8+ T-cells was demonstrated in patients of category 2 and 3 (BAL within, respectively, 2–7 and 8–30 days after termination of antigen exposure), this tendency continued in category 4 (BAL within 1–12 months after antigen exposure), which is in agreement with TRENTIN *et al.* [17]. This might also explain the course of the CD4+/CD8+ ratio, which still decreased after challenge, and returned to the normal range starting 1 month after the last exposure. Thus, the timing of observation is an important variable accounting for the heterogeneous data reported from different authors.

The presence of plasma cells in BALF suggests recent antigen exposure and, as such, EAA [28, 29]. Following an initial increase, more than a week after the last antigen exposure, both the number of plasma cells and Ig levels were found to decrease, indicating a relationship between plasma cells and Igs in BALF. Recently, REYNOLDS *et al.* [30] suggested the concept of local production of Igs within the lung after inhaling antigen, in addition to diffusion of Igs from the vessels. They also found significantly lower Ig levels in asymptomatic pigeon breeders. A rise in the concentrations of Igs was observed immediately after antigen exposure, parallelling the increase of plasma cells in BALF. However, Ig levels became maximum in category 2 (2–7 days following final antigen exposure). This may be due to the high molecular weight of Igs. Initially, Igs remain intravasculary and in the interstitium [31]. Bronchiolo-alveolitis in EAA caused by inflammation and antigenic stimulation is characterized by a rapid influx of PMNs into the alveolar space, just after challenge. PMNs are important mediators of tissue damage, and are able to enhance the permeability of capillary and alveolar membranes [32]. Due to this damage, Igs can easily diffuse across these no longer semipermeable membranes and enter the interstitium. The high levels of Igs and high numbers of plasma cells support the hypothesis that antibodies, presumably as antigen-antibody immune complexes, are involved in the pathogenesis of the early reaction in EAA [20, 30, 33–35].

The presence of high numbers of eosinophils in BALF, confirmed in the present study, is considered to be a parameter of a hypersensitivity reaction [2, 36] and occurs as part of the immune response to antigens [10, 36].

LAVIOLETTE *et al.* [26] found increased numbers of mast cells in BALF, parallel to lymphocytosis, in farmers without clinical symptoms. However, their role remains obscure [26, 37].

In summary, our findings demonstrate that interpretation of BALF analysis in EAA greatly depends on the time elapsed between final antigen exposure and the performance of BAL, and therefore affects the results of BALF analyses. Thus, no support was found for the existence of a so called "standard" BALF profile in EAA.

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References

1. Costabel U. – The alveolitis of hypersensitivity pneumonitis. *Eur Respir J* 1988; 1: 5–9.
2. Bosch van den JMM, Heye C, Wagenaar SjSc, Velzen-Blad van HCW. – Bronchoalveolar lavage in extrinsic allergic alveolitis. *Respiration* 1986; 49: 45–51.
3. Klech H, Hutter C. – Clinical guidelines and indications for bronchoalveolar lavage (BAL): Report of the European Society of Pneumonology Task Group on BAL. *Eur Respir J* 1990; 3: 937–974.
4. Johnson MA, Nemeth A, Condez A, Clarke SW, Poulter LW. – Cell-mediated immunity in pigeon breeders' lung: the effect of removal exposure. *Eur Respir J* 1989; 2: 445–450.
5. Fournier E, Tonnel AB, Gosset Ph, Wallaert B, Ameisen JC, Voisin C. – Early neutrophil alveolitis after antigen inhalation in hypersensitivity pneumonitis. *Chest* 1985; 88: 563–567.
6. Rust M, Schultze-Werninghaus G, Meier-Sydow J. – Bronchoalveolar lavage as a tool to assess an inhalative provocation in extrinsic allergic alveolitis. *Prax Klin Pneumol* 1986; 40: 229–232.
7. Milburn HJ. – Lymphocyte subsets in hypersensitivity pneumonitis. *Eur Respir J* 1992; 5: 5–7.
8. Mornex JF, Cordier G, Pages J, *et al.* – Activated lung lymphocytes in hypersensitivity pneumonitis. *J Allergy Clin Immunol* 1984; 74: 719–728.
9. Dugas M, Wallaert B, Tonnel A-B, Voisin C. – From subclinical alveolitis to granulomatosis. *Chest* 1989; 96: 931–933.
10. Hance AJ, Saltini C, Crystal RG. – Does de novo immunoglobulin synthesis occur on the epithelial surface of the human lower respiratory tract? *Am Rev Respir Dis* 1988; 137: 17–24.
11. Reynolds SP, Jones KP, Eduards JH, Davies BH. – Immunoregulatory proteins in bronchoalveolar lavage fluid. A comparative analysis of pigeon breeders' disease, sarcoidosis and idiopathic pulmonary fibrosis. *Sarcoidosis* 1989; 6: 125–134.
12. Calvanico NJ, Ambegaonkar SP, Schlueter DP, Fink JN. – Immunoglobulin levels in bronchoalveolar lavage fluid from pigeon breeders. *J Lab Clin Med* 1980; 96: 129–140.
13. Ojanen T, Terho EO, Tukiainen H, Mäntyjärvi RA. – Class-specific antibodies during follow-up of patients with farmer's lung. *Eur Respir J* 1990; 3: 257–260.
14. Dall'Aglio PP, Pesci A, Bertorelli G, Brianti E, Scarpa S. – Study of immune complexes in bronchoalveolar lavage fluids. *Respiration* 1988; 54: 36–41.
15. Kelley J. – Cytokines of the lung. *Am Rev Respir Dis* 1990; 141: 765–788.
16. Haslam PL, Dewar A, Butchers P, Primett ZS, Newman-Taylor A, Turner-Warwick M. – Mast cells, atypical lymphocytes, and neutrophils in bronchoalveolar lavage in extrinsic allergic alveolitis. *Am Rev Respir Dis* 1987; 135: 35–47.
17. Trentin L, Marcer G, Chilosi M, *et al.* – Longitudinal study of alveolitis in hypersensitivity pneumonitis patients: an immunological evaluation. *J Allergy Clin Immunol* 1988; 82: 577–585.
18. Haslam PL. – Bronchoalveolar lavage in extrinsic allergic alveolitis. *Eur Respir J* 1987; 154: 120–135.
19. Keller RH, Swartz S, Schlueter DP, Bar-Sela S, Fink JN. – Immunoregulation in hypersensitivity pneumonitis: phenotypic and functional studies of bronchoalveolar lavage lymphocytes. *Am Rev Respir Dis* 1984; 130: 766–771.
20. Semenzato G, Trentin L. – Cellular immune responses in the lung of hypersensitivity pneumonitis. *Eur Respir J* 1990; 3: 357–359.
21. Carter P. – Ultramicroestimation of human serum albumin: binding of the cationic dye, 5,5'-dibromo-o-cresolsulfonphthalein. *Microchem J* 1970; 15: 531–539.
22. Louderback A, Measley A, Taylor NA. – A new dye-binder technique using bromocresol purple for determination of albumin in serum. *Clin Chem* 1968; 14: 793–794.
23. Cormier Y, Bélanger J, Laviolette M. – Persistent bronchoalveolar lymphocytosis in asymptomatic farmers. *Am Rev Respir Dis* 1986; 133: 843–847.
24. Salvaggio JE. – Immune reactions in allergic alveolitis. *Eur Respir J* 1991; 4, (Suppl. 13): 47s–59s.
25. Nakajima H, Iwamoto I, Tomoe S, *et al.* – CD4+ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. *Am Rev Respir Dis* 1992; 146: 374–377.
26. Laviolette M, Cormier Y, Loiseau A, Soler P, Leblanc P, Hance AJ. – Bronchoalveolar mast cells in normal farmers and subjects with farmer's lung. Diagnostic, prognostic, and physiologic significance. *Am Rev Respir Dis* 1991; 144: 855–860.
27. Rose C, King TE. – Controversies in hypersensitivity pneumonitis. *Am Rev Respir Dis* 1992; 145: 1–2.
28. Costabel U, Bross KJ, Guzman J, Matthys H. – Plasmazellen und Lymphozytensubpopulationen in der bronchoalveolären Lavage bei exogen-allergischer Alveolitis. *Prax Klin Pneumol* 1985; 39: 925–926.
29. Drent M, Velzen-Blad van H, Daimant M, *et al.* – Differential diagnostic value of plasma cells in bronchoalveolar lavage fluid. *Chest* 1993; 103: 1720–1724.
30. Reynolds SP, Edwards JH, Jones KP, Davies BH. – Immunoglobulin and antibody levels in bronchoalveolar lavage fluid from symptomatic and asymptomatic pigeon breeders. *Clin Exp Immunol* 1991; 86: 278–285.
31. Rosen FS, Cooper MD, Wedgwood RJP. – The primary immunodeficiencies. *N Engl J Med* 1984; 311: 235–242.
32. Kaltreider HB. – Phagocytic, antibody and cell-mediated immune mechanisms. *In:* Murray JF, Nadel JA, eds. Textbook of respiratory medicine. Philadelphia, Saunders, 1988; pp. 332–357.
33. Pesci A, Bertorelli G, Dall'Aglio PP, Neri GP, Olivieri D. – Evidence in bronchoalveolar lavage for third type immune reactions in hypersensitivity pneumonitis. *Eur Respir J* 1990; 3: 359–361.
34. Sibille Y, Martinot JB, Staquet P, Delaunois L, Chatelain B, Delacroix DL. – Antiproteases are increased in bronchoalveolar lavage in interstitial lung disease. *Eur Respir J* 1988; 1: 498–504.
35. Bice DE, Muggenburg BA. – Localized immune memory in the lung. *Am Rev Respir Dis* 1988; 138: 165–171.
36. Yamaguchi E, Saito S, Okazaki N, Abe S, Kawakami Y. – Plasma cells in the bronchoalveolar lavage fluid of a patient with eosinophilic pneumonia. Morphologic proof of local production of antibodies. *Chest* 1988; 93 110–113.
37. Heard BE, Nunn AJ, Kay AB. – Mast cells in human lungs. *J Pathol* 1989; 157: 59–63.