EXPRESSION OF THE Fc-RECEPTOR FOR IgE (FcεRII, CD23) ON ALVEOLAR MACROPHAGES IN EXTRINSIC ALLERGIC ALVEOLITIS

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Extrinsic allergic alveolitis (EAA)[1] or hypersensitivity pneumonitis is caused by exposure to allergens derived, for instance, from pigeon and parakeets or from actinomycetes found in moldy hay (1, 2). Among the possible immunological mechanisms involved in EAA, T cell–mediated reactions are discussed (3), but in general, EAA is considered a type III immune complex disease (4, 5). Immune complex deposits may activate complement and initiate inflammation with recruitment of granulocytes (6). While deposition of immunoglobulin and of complement can be detected in histology (7), there is, however, no consistent decrease of plasma complement levels observed in patients with EAA (8).

An involvement of an IgE response until recently appeared unlikely since prick tests were found negative and no specific IgE was detectable in serum of patients with EAA (4). On the other hand, bird antigens in principle are able to induce an IgE response as demonstrated in asthmatic bird fanciers (9). Based on a frequently observed obstructive component similar to allergic asthma and the sometimes immediate onset of clinical symptoms in EAA, we hypothesized that IgE might be involved in EAA, but might be operative only locally in the bronchoalveolar space. In fact, a role for IgE in EAA was suggested by studies demonstrating histamine release from bronchoalveolar lavage cells after addition of anti-IgE (10). Since IgE antibodies are only effective when bound to specific Fc-receptors on the surface of leukocytes, we investigated the expression of such FcR on alveolar macrophages (AM) in patients with EAA. As demonstrated herein, there is a strong upregulation of the FcεRII in such patients. Furthermore, these FcRs appear to be partially occupied by endogenous IgE, suggesting that IgE-mediated activation of alveolar macrophages may be involved in pathophysiology of EAA.

Materials and Methods

10 untreated patients with EAA (7 women, 3 men; mean age 43; range between 23 and 66 yr) were investigated. The diagnosis was based on a history of antigen exposure, typical clinical features (dyspnea, cough, chills, weight loss, fever), diffuse opacities in chest x-ray, and the functional pattern of interstitial lung disease. Pulmonary function tests included dynamic spirometry, flow volume spirometry, blood gas analysis, and single-breath diffusing capacity (DLco). All patients had precipitating antibodies against the relevant antigens as determined in Ouchterlony assays kindly provided by Dr. de Haller, Geneva, Switzerland. Time between last antigen exposure and examination ranged between 1 and 4 d.

For comparison we investigated 10 patients with pulmonary sarcoidosis (5 men, 5 women; mean age 29; range between 21 and 48). Diagnosis was confirmed by histological examination of transbronchial biopsies.

As controls 6 healthy volunteers (5 women, 1 man; mean age 26; range between 24 and 29 years) were examined. Clinical evaluation showed no pulmonary abnormalities. All patients and controls were nonsmokers.

After informed consent was obtained, all persons underwent fiberoptic bronchoscopy and bronchoalveolar lavage (BAL). Lavage was performed by instillating 160 ml 0.9% saline solution in 20-ml aliquots into lingula or middle lobe and withdrawn by syringe. Total cells were counted and cytocentrifuge smears were prepared for cytological and immunocytochemical analysis. Differential counts of 100 cells were made (Wright-Giemsa staining). For immunocytochemical staining mAbs were used in conjunction with the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique (11). T cell subsets were identified with $CD4^+$ and $CD8^+$ mAbs (Dakopatts, Hamburg, FRG). For identification of the Fc_ϵ-receptor ($Fc_\epsilon R$) on alveolar macrophages, the mAb M-L25 (CD23) (11) was used. In addition, the $Fc_\epsilon R$ was identified with preformed IgE immune complexes consisting of a chimeric IgE with a human IgE heavy chain and a murine NIP-specific variable region and of NIP (nitrophenyl-) ovalbumin as antigen. Binding of the complex was then detected with the murine anti-NIP mAb. Endogenous IgE bound to the AM was detected with the mAb M-E 567 (11). For all stainings the IgG_1 $CD8^+$ mAb $M-T8_{11}$ was used as isotype control. The slides were analyzed visually by light microscopy and a total of 100 cells were counted, in that for CD4/CD8 determination only small cells (<10 μm) and for analysis IgE and IgE receptor expression only large cells (>12 μm) were evaluated.

For cytometry images of 50–100 cells per specimen, we used a microscope (Zeiss Axiomat, objective magnification ×25) and TV camera (Bosch T1VK9B1). Each cell was segmented semiautomatically or interactively and was measured with an IBAS system (Kontron) interfaced to a Microvax computer (Digital Equipment) and the image analysis system BASISS (12). The OD readings were corrected by substracting the background of the respective slide. The OD values for all cells were allocated to one of 20 channels on a linear scale and distributions of intensity versus cell number were plotted. Percent positive cells were determined using channel-by-channel subtraction of isotype control histograms from histograms for specific staining. Mean specific staining intensity was determined by substracting the mean OD for the control staining from the mean OD for the specific M-L25 staining.

Results and Discussion

Five of the EAA patients studied were found to be reactive to thermophilic actinomycetes (Farmer's lung) and five to avian proteins (bird fancier's lung), as evidenced by detection of specific IgG antibodies. The typical clinical symptoms such as fever and dyspnea as well as impairment of lung function (PO_2 at rest <70 mm Hg, DL_{co} <75%) were present in every patient. Bronchoalveolar lavage showed an increased percentage of lymphocytes with predominance of $CD8^+$ cells in most samples. Still, with the increased recovery of BAL cells in EAA compared with control donors there is a more than 10-fold increase in the number of alveolar macrophages as well (Table I).

TABLE I

Cellular Composition of Bronchoalveolar Lavage

Subjects	Sex	Age	Total cell count	Alveolar macro-phages	Lympho-cytes	Neutro-phils	Eosino-phils	T4/T8 ratio
			$\times$ 10^6	%	%	%	%	
EAA 1	M	66	19.1	70	20	4	6	0.8
2	F	51	15.0	46	45	2	7	4.0
3	M	35	12.7	40	16	17	25	0.3
4	F	53	14.5	62	31	2	5	0.6
5	F	38	25.1	43	35	0	22	0.4
6	F	57	19.5	29	61	1	9	0.6
7	F	49	12.3	28	70	0	2	0.5
8	F	24	16.3	34	60	2	2	0.6
9	M	23	32.0	46	48	1	5	0.3
10	F	39	28.8	51	25	10	6	0.8
x ± SD	7 F, 3 M	43 ± 13	19 ± 6.5	45 ± 13	41 ± 17	4 ± 5	9 ± 7	0.9 ± 1.0
Sarcoidosis (n = 10) x ± SD	5 F, 5 M	29 ± 10	8.2 ± 2.0	73 ± 11	23 ± 8	3 ± 2	2 ± 1	2.1 ± 0.4
Controls (n = 6) x ± SD	5 F, 1 M	26 ± 2	0.8 ± 0.2	93 ± 2	3 ± 1	3 ± 1	1 ± 1	ND

Incubation of the BAL cells with the anti Fc_ϵ-receptor antibody M-L25 gave no significant staining in controls and in patients with sarcoidosis. By contrast, a strong staining of alveolar macrophages but not of lymphocytes was detected in EAA patients (Fig. 1), while the control antibody $CD8^+$ stained only a portion of the lymphocytes but not the alveolar macrophages.

Microscopic inspection by eye revealed that an average of 73% of alveolar macrophages were positive for Fc_ϵ-receptor. In cytometry the evaluation of specific optical density using digital image analysis demonstrates a monomodal distribution of staining intensity (Fig. 2). Determination of the percentage of positive cells largely confirmed the data from visual analysis in that an average of 71.8% of AM was found to be positive for M-L25 in EAA, but no significant staining was seen in sarcoidosis or in controls (Table II). Cytometry, however, revealed a lower percentage of positive cells in one case (Case 4), where an overlap of control staining and of specific staining rendered enumeration by eye difficult.

While specific optical density for M-L25 staining was between 0.05 and 0.15 for the majority of EAA patients, one patient (Case 8, Table II) had an extremely high value with a specific OD of 0.265. This patient exhibited a more severe clinical course with repeated attacks during the last years. At the time of bronchoscopy she had fever and orthopnea. Chest x-ray showed massive diffuse infiltrations and lung function was strongly impaired in this patient. Based on this observation, we may speculate that the degree of $Fc_\epsilon R$ expression correlates with the severity of clinical symptoms.
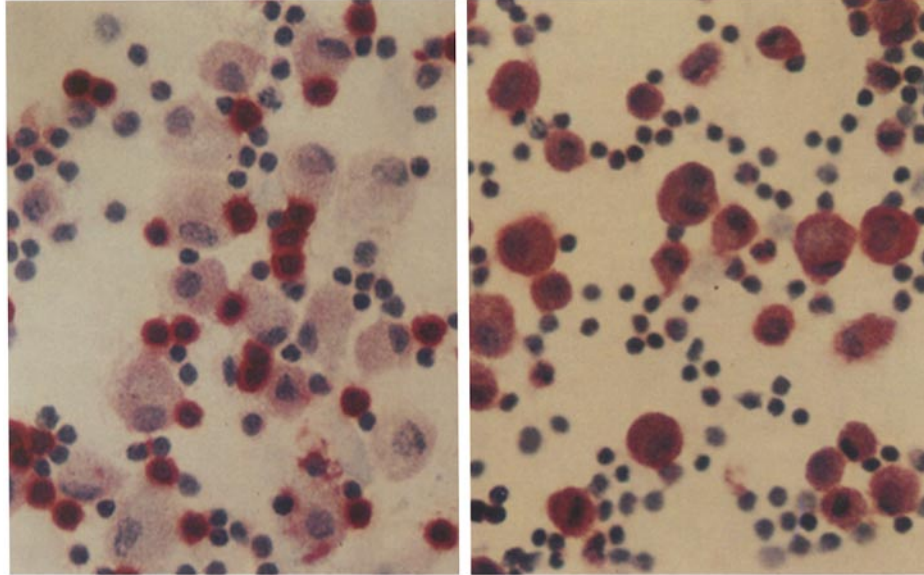
FIGURE 1.   Fc$_\epsilon$-receptor expression on AMs in exogenous allergic alveolitis. Cytospin preparations of BAL cells from patient 9 were stained with the anti-Fc$_\epsilon$RII (CD23) mAb M-L25 (*right*) and as isotype control with the CD8 mAb M-T811 (*left*). M-L25 strongly stains the (large) AM, while M-T811 recognizes part of the (small) lymphocytes, but no staining is observed in the AMs with this reagent.

AM in EAA appear to be the major cells with upregulation of Fc$_\epsilon$RII since no significant staining of lymphoid cells in BAL was detected with M-L25. Furthermore, this process appears to be restricted to the local site since in preliminary experiments no staining of peripheral blood monocytes with M-L25 was detected in EAA patients (data not shown).

To confirm the results with the CD23 antibody, Fc$_\epsilon$Rs were also analyzed with preformed IgE immune complexes. Results in patients 2, 6, 7, and in three addi-
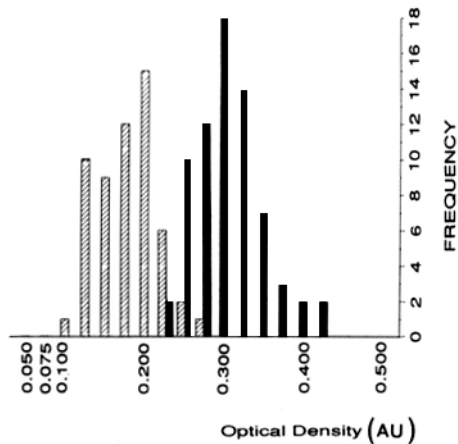

Optical Density (AU)

FIGURE 2.   Evaluation of Fc$_\epsilon$-receptor expression by cytometry. The optical density of samples stained with the anti-Fc$_\epsilon$RII (CD23) mAb M-L25 or with the CD8 mAb M-T811 was evaluated with a digital image analysis system by gating on the large (>12 μm) cells only. Cells were allocated to one of 20 channels on a linear scale. Dark bars, M-L25 staining; open bars, M-T811 control staining. The percentage of positive AM in this example was 95%, the mean specific staining intensity, i.e., mean channel for M-L25 minus mean channel for M-T811, was 0.120 (patient 5).

TABLE II
FcεR Expression on AM in Exogenous Allergic Alveolitis

Subjects	Fcε-RII (CD23) Expression			FcεR (IgE-anti-NIP)* expression	Endogenous IgE
	Visual‡ determination	Cytometric determination		Visual‡ determination	Visual‡ determination
		Specific	OD		
	%	%	AU§	%	%
EAA 1	71	59.0	0.116		
2	70	74.3	0.113	62	65
3	78	85.6	0.115		
4	64	32.8	0.050		59
5	77	91.8	0.127		
6	65	69.5	0.090	64	
7	72	53.7	0.096	69	68
8	81	92.6	0.265		75
9	68	70.5	0.151		
10	83	87.7	0.114		
x ± SD	73 ± 6	71.8 ± 19.2	0.123 ± 0.053	65 ± 3‖	66 ± 6
Sarcoidosis (n = 10)					
x ± SD	0	1 ± 0	−0.006 ± 0.011¶	ND	0 (n = 4)
Controls (n = 6)					
x ± SD	0	0	−0.004 ± 0.006¶	0 (n = 4)	0 (n = 6)

Cells spun onto glass slides were reacted with the different mAbs antibodies and stained with APAAP technology. Both for visual evaluation and for cytometry only large (>12 μm) AMs were analyzed.

* IgE-anti-NIP, preformed immune complexes consisting of a chimeric antibody with mouse anti-NIP binding region and human IgE Fc-portion plus NIP albumin.

‡ Only cells with a circular staining were considered positive, while slight changes of diffuse granular staining were not evaluated.

§ AU, arbitrary units.

‖ Binding of IgE anti-NIP immune complexes was demonstrated in three additional EAA patients (x = 65 ± 6%).

¶ Negative values in sarcoidosis and in controls result from the slightly higher background staining with the CD8 control antibody compared with CD23 antibody.

tional patients (Table II and footnote) revealed strong circular staining of the AM in EAA with again >60% positive cells, while in the apparently healthy controls only a slightly increased diffuse granular staining was observed and no cells were found positive (Table II, column 5).

Finally, we asked whether binding of endogenous IgE to the FcεR can be detected on the AM of EAA patients. In fact, staining with the anti–human IgE mAb M-E 567 demonstrated positivity in four EAA patients but not in control donors (Table II, column 6). We do not know whether the IgE is allergen specific. This important question would require sophisticated technology in order to discriminate specific cell-bound IgE and specific cell-bound IgG. If it were specific IgE, we would have to assume local production of this immunoglobulin (13) in order to explain preferential binding of specific over other IgE. Our prominent finding, i.e., the strong staining with the CD23 antibody M-L25 on AM in 10 of 10 patients with EAA but not in sarcoidosis or controls, would be consistent with an IgE-mediated process. Since

IL-4, a T cell–derived cytokine, is a potent inducer of IgE production and of FcεRII expression on monocytes (14, 15), we may speculate that this cytokine is produced after allergen exposure in EAA, followed by induction of IgE and IgE receptors on AM. The IgE receptor may be shed and function as regulatory IgE binding factor (16), but more importantly, FcεRII on the cell surface when loaded with IgE may transduce an activating signal after crosslinking by allergen (17). The activated AM may then produce a host of inflammatory mediators including AA metabolites and cytokines. This pathophysiological concept for EAA, based on the finding of increased FcεRs is, however, not likely to be the only mechanism and it may be speculated that the immune complex–mediated mechanisms and IgE-induced mechanisms may operate in parallel.

In addition to providing a clue for the explanation of pathophysiology in EAA, the finding of an upregulated FcεRII in EAA may be of diagnostic value in that it may substantiate the allergic etiology of an ill-defined interstitial lung disease, and furthermore, it may assist in monitoring the clinical course of EAA.

Summary

Expression of the Fc receptor for IgE (FcεR) was analyzed on alveolar macrophages (AM) in 10 patients with extrinsic allergic alveolitis (EAA) compared with 10 patients with sarcoidosis and to 6 apparently healthy controls. By using the anti-FcεRII mAb M-L25 in immunocytochemistry experiments, we found that >60% of AM in 10 of 10 patients with EAA were strongly positive, as evidenced by visual analysis in light microscopy and by cytometry. By contrast, no significant staining was detected in sarcoidosis or in controls with either method. Similar results were obtained when FcεR were identified with preformed immune complexes consisting of NIP-specific human/mouse chimeric IgE antibody plus NIP-ovalbumin. Furthermore, >60% of AM in patients with EAA stained positive for IgE, demonstrating that endogenous IgE is bound to the AM.

Our data suggest that IgE antibodies bound to FcεRII on AM may be involved in pathophysiology of extrinsic allergic alveolitis by activation of the AM after binding of allergen to the cell surface IgE. Furthermore, with the clearcut pattern of FcεRII expression in extrinsic allergic alveolitis it may be possible to use CD23 antibodies for differential diagnosis of inflammatory lung disease.

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