

Antigen-specific IgG antibodies in feather duvet lung

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ABSTRACT

Background Feather duvet lung (FDL) is a rare subgroup of bird fancier's lung (BFL). We were interested in determining antigen-specific IgG antibodies in patients with FDL and comparing them with those with BFL.

Material and methods Specific IgG antibodies against goose and duck feathers, analysed with an automated fluorimetric enzyme immunoassay, were measured in healthy subjects (group A, $n = 30$), in patients with FDL (group B, $n = 10$) and with BFL (group C, $n = 35$); typical specific IgG antibodies of BFL in groups B and C.

Results An optimal threshold value for antibodies against goose or duck feathers to differentiate patients with either BFL or FDL from healthy subjects was determined at 10.85 mg L^{-1} for goose feathers and at 8.81 mg L^{-1} for duck feathers, respectively. Specific IgG antibodies against goose feathers were significantly higher in group B compared with group C. A ratio of specific IgG antibodies against goose feathers and budgerigar antigens with a threshold value of 0.91 could discriminate between patients with FDL and BFL with a specificity of 97% and a sensitivity of 90%.

Conclusions We were able to demonstrate the significant difference in IgG antibodies in patients with FDL and BFL and their contribution to discriminate between these similar kinds of extrinsic allergic alveolitis.

Keywords Bird fancier's lung, duck feathers, extrinsic-allergic alveolitis, feather duvet lung, goose feathers, hypersensitivity pneumonitis, specific IgG antibodies.

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Introduction

Extrinsic allergic alveolitis (EAA), also known as hypersensitivity pneumonitis, is a diffuse granulomatous lung disease caused by inhalation of various antigens [1]. Probably the most common form of EAA is bird fancier's lung (BFL), caused by exposure to budgerigars, pigeons, parrots or other small caged birds [2]. Antigen sources are bird excrement, feathers and serum [3]. There are several recommended diagnostic criteria for EAA, which all include the detection of antigen-specific IgG antibodies [4–7]. A positive antigen-specific IgG antibody is one of the most significant predictors for the clinical diagnosis of EAA [8].

There are different methods to test antigen-specific IgG antibodies. Testing for precipitins with double diffusion is only a qualitative test and without high sensitivity. Cases of undiagnosed EAA have been attributed to improper quality control [9]. A quantitative automated fluorimetric enzyme immunoassay (FEIA) has been tested in BFL and provides an international standard for EAA serology. Increasing antibody titres reflected

the likelihood of EAA, and decreasing titres confirmed antigen avoidance [10]. Cross-reactions are frequently observed in testing antigen-specific IgG antibodies between different kinds of birds in sera of patients with BFL [11,12].

Feather duvet lung (FDL) is a rare case of BFL caused by feathers from duvets or pillows [13]. Strongly positive specific IgG antibodies against goose feathers can be detected [14]. In patients with chronic BFL an acute exacerbation caused by the use of a feather duvet is possible [15].

The aim of this study was to determine and compare specific IgG antibodies in patients with FDL and BFL and in healthy subjects. We were interested to know whether these antibodies can help to discriminate between the two similar groups of EAA. To the best of the authors' knowledge, there are no reports about quantitative specific IgG antibodies against goose and duck feathers in patients with FDL and possible cross-reactions of these serum antibodies in patients with BFL.

Material and methods

Diagnosis of EAA

The diagnostic criteria for EAA included (i) a definite history of bird keeping at home or using feather duvets and/or pillows, (ii) recurrent episodes of symptoms compatible with EAA, (iii) elevated/positive specific IgG antibodies to avian antigens, (iv) inspiratory crackles on physical examination, (v) findings compatible with EAA on chest radiograph or high resolution CT scan, (vi) decreased diffusing capacity and/or arterial hypoxemia, either at rest or on exercise, (vii) a lymphocytic alveolitis in BAL fluid, (viii) pulmonary histological changes compatible with EAA, (ix) a positive inhalative challenge and (x) a positive avoidance test. The diagnosis was confirmed if the patient fulfilled six of the 10 criteria [7].

Study groups

Group A: Serum samples were obtained from healthy subjects with no clinical history of EAA or other pulmonary diseases ($n = 30$).

Group B: Serum samples were retrospectively reviewed from patients with a clinical diagnosis of FDL ($n = 10$). All were diagnosed in the Fachkrankenhaus Coswig between April 2005 and September 2008.

All patients had a definite history of feather duvets or pillows causing the clinical signs of EAA. Only two of them had a history of a bird (budgerigar) at home, but this was at least 10 years before the first clinical signs of EAA started. Five subjects grew up in a rural area with their own goose farms, two of them had clinical signs of fever and dyspnoea while plucking goose feathers in their childhood. Four subjects used raw feathers from their own geese to fill their duvets and pillows. In one subject with a chronic course of EAA an inhalative challenge with goose feathers from their own pillow was carried out to verify the diagnosis. The complete clinical data of the 10 patients with FDL were already published as a major part of a study about presenting features of FDL [16].

Group C: Serum samples were retrospectively reviewed from patients with a clinical diagnosis of BFL ($n = 35$). All were diagnosed or treated in the Fachkrankenhaus Coswig between April 2005 and September 2008. Even when there is no detailed information about the quality of bird exposure of each patient, caged budgerigars are the most frequent, followed by pigeons (pigeon breeding) and parrots.

Detection of serum antibodies with automated fluorimetry

A solid-phase indirect FEIA automated system was used according to the manufacture's instructions (UniCAP 100; Sweden Diagnostics (UK) Ltd, Milton Keynes, UK). The antigens (code reference) were goose feathers (e70), duck feathers (e86),

budgerigar antigen-mix (Ge90), pigeon antigen-mix (Ge91) and parrot antigen-mix (Ge92). Each of the antigen-mixes from budgerigars, pigeons and parrots consists of antigens from feathers, serum and droppings.

In all three groups specific IgG antibodies against goose and duck feathers were analysed, additionally, in group B and C antibodies against budgerigar antigen-mix, pigeon antigen-mix, and parrot antigen-mix were analysed. In group A, specific antibodies against the last three antigens were not examined, because threshold values for these antibodies in healthy subjects already exist.

In group B and C the whole panel of specific antibodies was performed while the patients underwent the clinical diagnosis.

Statistical analysis

Statistical analysis was performed using statistical software SPSS (Version 14.01; SPSS Inc., Chicago, IL, USA). Data of the different antigen-specific IgG antibodies are presented as mean $\pm$ SD, the 95% confidence interval and the minimum/maximum values. A comparison of antigen-specific IgG antibodies against goose and duck feathers in the three different groups was performed using the Kruskal–Wallis-Test and pairwise Mann–Whitney-*U*-tests. *P*-values below 0.05 (global alpha) indicate a statistically significant difference. Bonferroni adjustment was applied to control alpha for multiple comparisons.

Logistic regression analysis was used to evaluate whether specific antibodies against goose and duck feathers could discriminate between healthy subjects (group A) and patients with an EAA (group B + C). To differentiate patients with either FDL or BFL from healthy subjects with optimal sensitivity and specificity, we estimated thresholds of specific antibodies against goose and duck feathers using Receiver Operating Characteristic Curves (ROC) and Youden's index. The area under the curve and their 95% confidence interval as a simple measure to compare the probability of a correct ranking of a (normal, abnormal) pair was calculated.

Although there were cross-reactions of the investigated specific IgG antigens in both clinical groups (B + C), we expected higher antigen-specific IgG antibodies against goose and duck feathers in group B and higher antibodies against budgerigar-mix, pigeon-mix and parrot-mix in group C. Therefore ratios of the antigen-values by two were performed and analysed with logistic regression and ROC analysis. Optimal cut points for discriminating patients with FDL (group B) on the one hand from patients with BFL (group C) on the other hand were determined.

Results

Clinical features

The mean age was 44 (25–56) years in the healthy control group (17 women and 13 men), 52 (26–68) years in patients with FDL

(all women) and 48 (22–72) years in patients with BFL (27 women and eight men). All of the patients with FDL were never-smokers. Among the patients with BFL, 32 were never-smokers and three former smokers. Of the patients with FDL, five had an acute, and five a chronic course of EAA, whereas among the patients with BFL 10 had an acute, and 25 a chronic course of EAA.

Laboratory findings

Mean values of the different antigen-specific IgG antibodies are displayed in Table 1. The mean value of antigen-specific IgG antibodies against goose feathers was statistically significantly higher in patients with FDL ($93.90 \pm 52.73 \text{ mg L}^{-1}$) when compared with either the BFL group [$57.57 \pm 39.85 \text{ mg L}^{-1}$; $P = 0.047$ (without Bonferroni adjustment of global alpha)] or the healthy control subjects ($5.11 \pm 1.94 \text{ mg L}^{-1}$; $P < 0.001$). The difference between patients with BFL and the healthy control subjects was also statistically significant ($P < 0.001$).

The mean value of antigen-specific IgG antibodies against duck feathers was not statistically significantly different between the patients with FDL ($48.40 \pm 22.28 \text{ mg L}^{-1}$) and BFL ($34.83 \pm 25.82 \text{ mg L}^{-1}$; $P = 0.073$), but in both clinical groups it was statistically significantly higher than in healthy control subjects ($4.92 \pm 1.91 \text{ mg L}^{-1}$; $P < 0.001$) (Fig. 1a,b).

An optimal threshold value for antigen-specific IgG antibodies against goose or duck feathers to differentiate between patients with either BFL or FDL and from healthy subjects was determined for goose feathers at 10.85 mg L^{-1} (specificity

100%, sensitivity 97.8) and for duck feathers at 8.81 mg L^{-1} (specificity 100%, sensitivity 91.1, Table 2).

To discriminate patients with FDL from those with BFL, we analysed antigen-specific IgG antibodies against goose or duck feathers, against budgerigar, pigeon or parrot antigen-mix and ratios of them (goose or duck feathers divided by budgerigar, pigeon, or parrot antigen-mix). We found a significant contribution only for the ratio of goose feathers/budgerigar antigen-mix ($P = 0.012$), and not for the others. An optimal threshold value for the ratio of goose feathers/budgerigar antigen-mix was 0.91 with a specificity of 97% and a sensitivity of 90%. Lowering the threshold value to 0.78 will cause the sensitivity to increase to 100%, but the specificity will decrease to 74%.

Discussion

In this study, we found a threshold value to discriminate healthy subjects from patients with EAA (either BFL or FDL) for antigen-specific IgG antibodies against goose feathers at 10.85 mg L^{-1} and for duck feathers at 8.81 mg L^{-1} using an automated FEIA.

There are different methods to detect antigen-specific IgG antibodies in EAA. These serum antibodies in EAA were first detected using the double diffusion test according to Ouchterlony in gel by visible precipitin lines, and are called precipitins [17]. This is, however, only a qualitative test with low sensitivity, possibly because of non-precipitating IgG antibodies [18]. Other tests for detecting antigen-specific IgG antibodies are

Table 1 Antigen-specific IgG antibodies measured with fluorimetric enzyme immunoassay against goose feathers, duck feathers, budgerigar antigen-mix, pigeon antigen-mix and parrot antigen-mix in healthy subjects (group A; $n = 30$), patients with feather duvet lung (group B; $n = 10$) and patients with bird fancier's lung (group C; $n = 35$)

IgG antibodies	Patient group	Mean	SD	95% Confidence interval	Minimum value	Maximum value
Goose feathers	A	5.11	1.94	4.38–5.83	2	10
	B	93.90	52.73	56.18–131.62	30	174
	C	57.57	39.85	43.88–71.26	7	154
Duck feathers	A	4.92	1.91	4.21–5.64	2	9
	B	48.40	22.28	32.46–64.34	22	86
	C	34.83	25.82	25.96–43.70	7	97
Budgerigar antigens	B	97.60	52.81	59.82–135.38	26	170
	C	109.89	68.28	86.43–133.35	10	200
Pigeon antigens	B	92.50	29.78	71.20–113.80	54	146
	C	94.83	61.07	73.85–115.81	12	200
Parrot antigens	B	105.30	64.49	59.17–151.43	35	200
	C	100.40	62.72	78.86–121.94	8	200

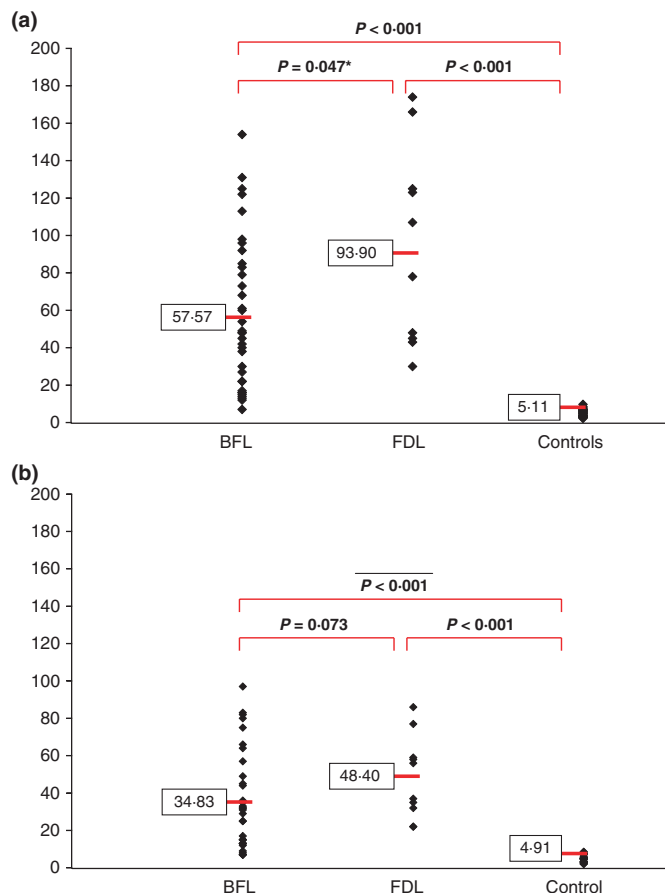


Figure 1 Comparison of mean values between patients with bird fancier's lung or feather duvet lung and controls. (a) Specific IgG antibodies against goose feathers (mg L^{-1}). *Only P -values ≤ 0.016 indicate statistically significant differences after control of the global alpha for multiple comparisons. (b) Specific IgG antibodies against duck feathers (mg L^{-1}).

described, such as an immunoblot assay [19], immunofluorescence technique (IFT) [20] or an enzyme immunoassay (ELISA) [21]. Antibodies should be determined quantitatively with methods such as ELISA or IFT because the higher the antibody titre, the more likely the allergic alveolitis [3]. *McSharry*

described in a pilot study a quantifying automated FEIA that provides for international standardization, thereby improving quality control. He measured the antibody activity in bird fanciers (with and without symptoms of EAA) and healthy control subjects using various avian antigens and compared them using different serological methods. Antigen-specific IgG antibodies, quantified by FEIA, provided a good discriminator of the disease and a threshold value of 10 mg L^{-1} was determined. Values below that limit were insignificant [10]. Corresponding to this study our threshold values for goose and duck feathers are in similar range.

The results of our study furthermore demonstrate that specific IgG antibodies against goose feathers are significantly higher in patients with FDL than that in patient with BFL although they are also elevated in BFL. Antibodies against duck feathers are elevated in FDL and BFL as well, but the difference in both groups was not significant.

Even if we believe that goose and duck feathers are specific antigens in FDL, two explanations are possible for the elevated antibodies against these antigens in patients with BFL. In sera from patients with BFL, detection of IgG antibodies against various types of birds is typical, not just the causative bird. In patients with BFL most sera react positively to all tested bird antigens such as those from pigeons, parakeets, canaries, chickens, ornamental finches, blackbirds and sparrows [12]. Some authors therefore recommend to just test the most common avian antigens from pigeons and budgerigars [11]. The reason for this cross-reactivity could be common antigens which are described in the albumins of various birds [22]. This cross-reactivity could likewise occur with the antigens of the feathers, particularly because the antigens in the dust of feathers seems to be most important in BFL [23].

Besides the cross-reactions, we believe that the high antibody levels against goose and duck feathers in patients with BFL are the original expression of antigen sensitization. There are reports about exacerbations of BFL caused by feather duvets. A man with chronic BFL caused by breeding budgerigars and hill mynas developed new symptoms after using a new feather duvet [15]. In a group of seven patients with FDL, one was previously diagnosed as having chronic BFL. He had an acute exacerbation after he used a feather duvet daily for the previous

Table 2 Discrimination between patients with extrinsic allergic alveolitis (feather duvet lung and bird fancier's lung) and healthy subjects

	P^*	Threshold (mg L^{-1})	Specificity (%)	Sensitivity (%)	AUC	95% CI of AUC
Goose feathers	0.014	10.85	100	97.8	0.997	0.99–1
Duck feathers	0.012	8.81	100	91.1	0.983	0.96–1

AUC, area under the curve.

*Logistic regression.

3 months [13]. It is without any doubt that feather duvets or pillows can be an antigen source for exacerbations of BFL and might be considered as the reason for an insidious course of chronic BFL after the 'causal' antigen avoidance. We would therefore recommend that patients with BFL should avoid contact with duvets or pillows.

Because of the above discussed cross-reactivity and the potential coincidental exposure to birds and feather duvets with elevated specific IgG antibodies to various avian antigens, discrimination of the real causative antigen source may be difficult. In the aforementioned study of *Inase* four of seven patients with FDL had a prior history of bird breeding [13]. In our 10 patients with FDL, only two had a history of a bird at home, but there had been at least 10 years before the first signs of clinical EAA. Because of the possible competitive antigen source of birds or feather duvets in a patient with both elevated antigen-specific IgG antibodies, we hypothesized that a ratio of these antigen-specific IgG antibodies could help to discriminate between patients with FDL and BFL. We found that only an antibody ratio of goose feathers and budgerigar antigen-mix was important in discriminating between the two clinical groups ($P = 0.012$). Using a threshold value of 0.91, discrimination between patients with FDL and BFL could be performed with a specificity of 97% and a sensitivity of 90%. If the threshold value is decreased to 0.78 the sensitivity will increase to 100%, but at the expense of a lower specificity of 74%. That means that a ratio above 0.91 is highly suspicious for FDL. There is no significance in the other antibody ratios such as goose feathers/pigeon-mix, goose feathers/parrot-mix, duck feathers/budgerigar-mix, duck feathers/pigeon-mix or duck feathers/parrot-mix. We explained this with the mainly goose feather-filled feather duvets and that budgerigar breeding is the main antigen source of BFL in our region.

A limit of our study was that we did not distinguish in the healthy control group between those with or without contact to duvets or pillows. In farmers we know that positive precipitins to moulds or thermoactinomycetes are a sign of sensitization and not of definitely having EAA [24]. In contrast to the specific exposure to organic dust in farming, we believe that a further discrimination of our control group is not necessary. On the one hand the presence of duvets and pillows is ubiquitous in nearly all households, and on the other hand the antigenic powder particles of the feathers in geese and ducks are minimal [25]. The homogenous levels of antibodies below 10 mg L^{-1} in the control group of healthy subjects confirmed that further discrimination of the group was not necessary.

Another limitation is the retrospective observational design of our study, but because of the limited number of cases a prospective design was not possible. Only a few case reports exist about patients with FDL with a maximum case number of seven.

In conclusion, a threshold value for antigen-specific IgG antibodies for goose and duck feathers with a quantifying assay (FEIA) was determined. Goose feathers in particular are the specific antigen source of FDL, a rare subgroup of BFL. Additionally, this is a potential antigen source that might be overlooked by a patient with bird-related EAA following otherwise strict antigen avoidance measures. Even when antigen-specific IgG antibodies against goose feathers are highly increased in FDL and in BFL, caused by cross-reactivity or competitive antigen sources, they are significantly higher in FDL. If there are concurrent antigen sources an antibody ratio of goose feathers and budgerigar antigen-mix could help to determine the causative antigen source in cases of doubt.

Conflict of interest

There are no conflicts of interests for all authors.

Address

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