

Original

Involvement of Bird-related IgG Antibodies
in Interstitial PneumoniaKazuki Mashio*, Yasutsugu Fukushima*, Fumito Oomi, Takafumi Umetsu, Naruo Yoshida,
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SUMMARY

Background and Objective : Chronic interstitial pneumonia (IP) might include chronic hypersensitivity pneumonitis (HP) and chronic bird-related hypersensitivity pneumonitis (BRHP). A specific antigen is difficult to identify in these diseases, and such evidence would provide important clues suggesting a diagnosis of HP. In this study, we used an ImmunoCAP analysis system to measure specific IgG antibodies against pigeons and budgerigars in the sera of patients with IP and investigated the involvement of bird-related IgG antibodies in IP.

Methods : The study group comprised 22 patients with idiopathic pulmonary fibrosis (IPF), 8 with chronic IP, 7 with subacute HP, 7 with chronic HP, and 10 with control diseases. All cases were diagnosed from 2000 through 2011 at the Institute of Pulmonary Medicine and Clinical Immunology, Dokkyo Medical University. Clinical features, results of laboratory examinations, and levels of serum IgG antibodies against pigeons and budgerigars were compared.

Results : There were no significant differences among the disease groups in C-reactive protein, leukocyte count, lactate dehydrogenase, and the results of blood gas analysis. KL-6 and surfactant protein D were significantly higher in subacute HP and chronic HP. The levels of anti-pigeon IgG antibodies and anti-budgerigar IgG antibodies in each disease group were respectively as follows : IPF, 11.02 ± 5.97 mg/l, 5.03 ± 3.97 mg/l ; chronic IP, 10.04 ± 8.55 mg/l, 3.30 ± 1.47 mg/l ; subacute HP, 14.39 ± 9.13 mg/l, 7.96 ± 6.47 mg/l ; chronic HP, 24.97 ± 16.19 mg/l, 11.50 ± 13.80 mg/l ; and control diseases, 8.66 ± 3.15 mg/l, 3.77 ± 1.05 mg/l. The mean levels of anti-pigeon IgG antibodies and anti-budgerigar IgG antibodies were significantly higher in chronic HP. There was a positive correlation between anti-pigeon IgG antibodies and anti-budgerigar IgG antibodies ($R^2 = 0.715$, $p < 0.001$).

Conclusions : In patients with clinically diagnosed chronic HP, high levels of anti-pigeon IgG antibodies or anti-budgerigar IgG antibodies were confirmed using an ImmunoCAP analysis system. In general, HP (especially chronic HP) is difficult to diagnose definitively, and this analysis system is expected to facilitate diagnosis.

Key Words : idiopathic pulmonary fibrosis, chronic interstitial pneumonia, chronic hypersensitivity pneumonitis, bird-related hypersensitivity pneumonitis

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Mibu, Tochigi 321-0293, Japan.**Abbreviations** :

BAL = bronchoalveolar lavage

BRHP = bird-related hypersensitivity pneumonitis

HP = hypersensitivity pneumonitis

HRCT = high-resolution computed tomography

IIPs = idiopathic interstitial pneumonias
IP = interstitial pneumonia
IPF = idiopathic pulmonary fibrosis

INTRODUCTION

Hypersensitivity pneumonitis (HP) is an allergic lung disease (allergic alveolitis) caused by repeated inhalation of organic or inorganic dust antigens¹⁾ and is classified as acute, subacute or chronic²⁾. Specific antigens are difficult to identify, especially in chronic HP. During the course of disease, cough and dyspnea increase in association with irreversible progression of pulmonary fibrosis and emphysema. Previous studies have reported that chronic interstitial pneumonia (IP) may include chronic HP and chronic bird-related hypersensitivity pneumonitis (BRHP)³⁾. The clinical course of chronic HP is similar to that of idiopathic pulmonary fibrosis (IPF), and diagnosis on high-resolution computed tomography (HRCT) of the chest remains challenging. Chronic HP is also difficult to diagnose on microscopical examination of lung specimens obtained by surgical biopsy.

A variety of antigens, including fungi, bacteria, animal proteins, and chemical substances, can potentially cause chronic HP, and bird-associated antigens, such as bird droppings and feathers, are important⁴⁾. Proof of specific antibodies against these antigens would provide useful clues suggesting a diagnosis of HP. Antibody testing against bird-related antigens might be possible at special laboratories, but is still not widely performed clinically. In this study, we used an ImmunoCAP analysis system to measure specific IgG antibodies against pigeons and budgerigars in the sera of patients with IP^{5,6)} and investigated the involvement of bird-related IgG antibodies.

MATERIAL AND METHODS

Patients

The study group of this retrospective study comprised 22 patients with IPF, 8 with chronic IP, 7 with subacute HP, 7 with chronic HP, and 10 with control diseases (lung cancer, 4; pneumonia, 4; and chronic obstructive pulmonary disease [COPD], 2). All cases were diagnosed during the 12 years from 2000

through 2011 at the Institute of Pulmonary Medicine and Clinical Immunology, Dokkyo Medical University. Clinical data were obtained from patient records. Clinical features, results of laboratory examinations, and levels of serum IgG antibodies against pigeons and budgerigars were compared among the disease groups.

Major diagnostic criteria for IPF were as follows: (1) exclusion of known interstitial lung diseases such as collagen vascular disease, environmental exposure, and drug-induced pneumonitis; (2) restrictive impairment of pulmonary function and arterial blood gas exchange failure; and (3) bilateral reticular or ground-glass opacities accompanied by honeycombing in basal lung lesions on chest HRCT. Secondary diagnostic criteria were (1) an age of 50 years or older, (2) slow progression of respiratory problems, (3) a disease duration of 3 months or longer, and (4) fine crackles in both basal lung fields. A clinical diagnosis of IPF required that all three major diagnostic criteria and at least three of the four secondary diagnostic criteria were met. If surgical lung biopsy was performed and confirmed a usual interstitial pneumonia (UIP) pattern, the findings of honeycombing on chest HRCT were excluded from the major criteria.

Definition and diagnosis of chronic IP is based on the exclusion of known drug-induced pneumonitis, pneumoconiosis, environmental exposure, and collagen vascular disease associated with interstitial lung disease (ILD). If the diagnostic criteria for IPF or other idiopathic interstitial pneumonias (IIPs) are not met, chronic IP is diagnosed.

The diagnostic criteria for acute or subacute HP were as follows: (1) cough, shortness of breath, fever, fine crackles; (2) diffuse centrilobular granular or ground-glass opacities on chest HRCT, restrictive pulmonary function impairment, hypoxemia, evidence of inflammation on blood tests, lymphocytosis in bronchoalveolar lavage fluid (BALF), and a negative tuberculin reaction; (3) positive specific antibodies against trichosporon species (summer type HP); (4) symptom flare-up caused by home or occupational expo-

sure ; and (5) confirmation of granuloma, alveolitis, or Masson bodies on microscopical examination of a biopsy specimen of the lung, satisfying the diagnostic criteria for HP proposed by the Japanese Ministry of Health, Labour and Welfare⁷.

The diagnostic criteria for chronic HP were as follows : (1) environmental exposure to various antigens ; (2) fine crackles in chest ; (3) lymphocytosis in BALF or positive results of an induced lymphocyte proliferation test ; (4) diffuse centrilobular granular or ground-glass opacities on chest HRCT ; (5) pathological findings of chronic HP on surgical lung biopsy ; (6) clinical flare-ups caused by environmental exposure ; (7) progression of restrictive impairment of pulmonary function for more than 1 year or related symptoms lasting for 6 months or longer ; and (8) positive results of antigen inhalation provocation tests or improvement of symptoms in response to antigen avoidance.

Measurement of specific antibodies

We measured specific IgG antibodies against pigeons and budgerigars with the use of an ImmunoCAP analysis system (Phadia100 ; Phadia Inc., Uppsala, Sweden)^{5,6}. The antigens were pigeon serum (Ge93), droppings (e7), and feathers (Re215), and budgerigar serum (e79), feathers (e78), and droppings (e77). When all reagents were in excess, the fluorescence was proportional to the concentration of serum IgG antibody, and the titer was calculated by interpolation onto a 6-point standard curve generated by the fluorescence from standard samples of known IgG titer.

Statistical Analysis

Data are expressed as means \pm SD. Differences between groups were analyzed using nonparametric methods (Mann-Whitney *U* test). Spearman rank correlation coefficients were used to explore the relationship between the levels of serum IgG antibodies against pigeons and budgerigars. Clinical data analysis was performed with statistical software (JMP 10.0 ; SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as a *p* value of less than 0.05.

RESULTS

Characteristics of patients (Table 1)

The subjects were 22 patients with IPF (mean age, 65.8 years ; 16 men, 6 women), 8 with chronic IP (mean age, 67.5 years ; 3 men, 5 women), 7 with subacute HP (mean age, 53.9 years ; 3 men, 4 women), 7 with chronic HP (mean age, 63.4 years ; 4 men, 3 women), and 10 with control disease (mean age, 58.9 years ; 6 men, 4 women). In pulmonary-function testing, the mean value of VC% predicted was significantly higher in subacute HP than in IPF.

Laboratory findings at diagnosis (Table 2)

There was no significant difference among the disease groups in C-reactive protein, leukocyte count, lactate dehydrogenase, and the results of blood gas analysis. Levels of KL-6 and surfactant protein D (SPD) were significantly higher in subacute HP and chronic HP than in the other groups.

Measurement of antibody levels

The results of antibody levels against pigeons and budgerigars in each disease group are shown in Table 3. The mean value of anti-pigeon IgG antibodies was significantly elevated in chronic HP compared with other groups (Figure 1). The mean value of anti-budgerigar IgG antibodies was significantly elevated in chronic HP compared with chronic IP and control diseases (Figure 2).

Correlation between IgG against pigeons and IgG against budgerigars (Figure 3)

There was a positive correlation between anti-pigeon IgG antibody levels and anti-budgerigar IgG antibody levels ($R^2=0.715$, $p<0.001$).

DISCUSSION

Many interstitial lung diseases, including collagen vascular disease associated with ILD, pneumoconiosis, drug-induced pneumonitis, radiation pneumonitis, and chronic HP must be excluded in the differential diagnosis of "idiopathic" IP^{3,8~10}. In addition, some cases of chronic BRHP may be misdiagnosed as IIPs or chronic IP. We performed this study to attempt to identify factors useful for differential diagnosis.

Table 1 Characteristics of patients and baseline data

Characteristics	IPF n=22	CIP n=8	SHP n=7	CHP n=7	Control diseases n=10
Age, yr, median (range)	65.8 (47-79)	67.5 (54-80)	53.9 (39-66)	63.4 (57-76)	58.9 (30-78)
Male/female	16/6	3/5	3/4	4/3	6/4
Symptom duration, yr (range)	2.5±1.1 (1-5)	1.6±0.7 (1-3)	0.8±0.3 (0.5-1.2)	2±0.8 (1-3)	0.8±1.5 (0.1-5)
Ever smoker (%)	19 (86)	4 (50)	4 (57)	5 (71)	6 (60)
Antigen identified (%)	0 (0)	0 (0)	2 (29)	0 (0)	0 (0)
Bird fancier (%)	2 (9)	1 (13)	2 (29)	4 (57)	1 (10)
Use of feather bedclothes (%)	5 (23)	3 (38)	2 (29)	3 (43)	3 (30)
Physical findings					
Crackles (%)	20 (91)	6 (75)	3 (43)	6 (86)	2 (20)
Clubbed finger (%)	16 (73)	4 (50)	1 (14)	6 (86)	2 (20)
VC % predicted	66±17	83±29	92±16 *	79±13	85±33
FEV ₁ /FVC, %	113±12	114±10	104±12	110±8	93±17
DLco % predicted	55±11	64±12	60±12	60±16	89±39

Data are presented as mean ± SD or No. (%) unless otherwise indicated.

Definition of abbreviations : IPF, idiopathic pulmonary fibrosis ; CIP, chronic interstitial pneumonia ; SHP, subacute hypersensitivity pneumonitis ; CHP, chronic hypersensitivity pneumonitis ; FVC, forced vital capacity ; FEV₁, forced expiratory volume in 1 second ; DLco, diffusing capacity for carbon monoxide.

**p*<0.05 compared with IPF.

Table 2 Laboratory findings at diagnosis

Characteristics	IPF n=22	CIP n=8	SHP n=7	CHP n=7	Control diseases n=10
CRP (mg/dl)	0.61±0.56	0.70±0.73	0.65±0.60	0.37±0.34	2.34±3.14
WBC (/μl)	8753±2567	6686±1120	7357±2118	5400±395	7090±2353
LDH (IU/l)	259±43	245±33	279±41	291±51	242±127
KL-6 (U/ml)	1540±932	1869±1201	5926±3651 *†	6089±3791 *†	528±228
SPD (ng/ml)	202±100	114±38	470±326 *†	816±769 *†	134±77
PaO ₂ (Torr)	73.1±12.2	74.3±8.3	73.9±7.8	74.2±10.6	79.8±12.7
PaCO ₂ (Torr)	38.6±4.3	36.1±4.4	38.2±1.9	37.0±6.1	37.4±4.1

Data are presented as mean ± SD.

Definition of abbreviations : IPF, idiopathic pulmonary fibrosis ; CIP, chronic interstitial pneumonia ; SHP, subacute hypersensitivity pneumonitis ; CHP, chronic hypersensitivity pneumonitis ; CRP, C-reactive protein ; WBC, white blood cells ; LDH, lactate dehydrogenase ; KL-6, Krebs von den Lungen-6 ; SPD, surfactant protein D.

**p*<0.05 compared with IPF, †*p*<0.05 compared with CIP.

Table 3 Serum IgG antibodies against pigeons and budgerigars in IPF, CIP, SHP, CHP, and Control diseases

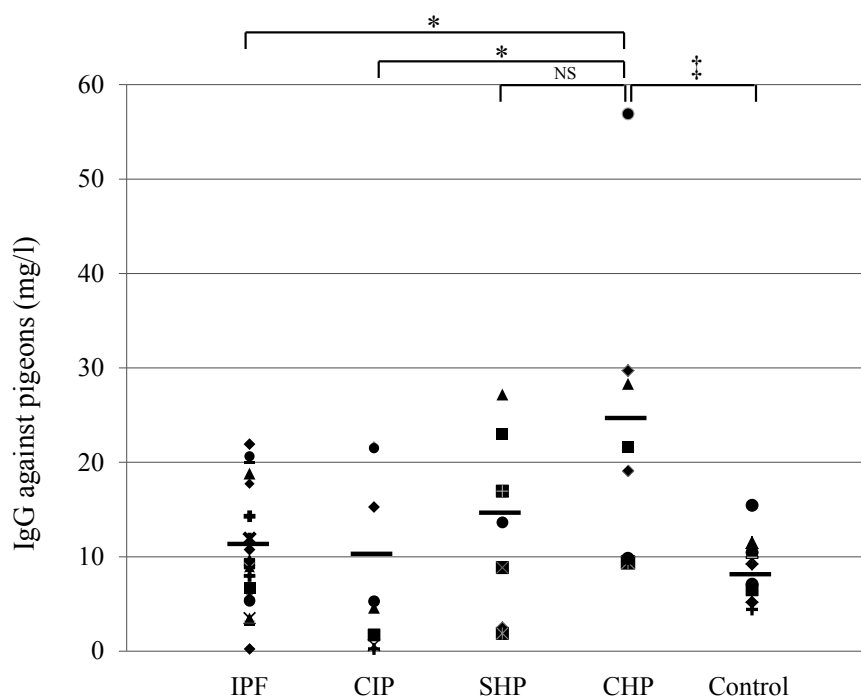
Serum IgG antibody	IPF n = 22	CIP n = 8	SHP n = 7	CHP n = 7	Control diseases n = 10
against pigeons (mg/l)	11.02 ± 5.97	10.04 ± 8.55	14.39 ± 9.13	24.97 ± 16.19* ^{†‡}	8.66 ± 3.15
against budgerigars (mg/l)	5.03 ± 3.97	3.30 ± 1.47	7.96 ± 6.47	11.50 ± 13.80 ^{†¶}	3.77 ± 1.05

Data are presented as mean ± SD.

For definition of abbreviations, see Table 1.

* $p < 0.05$ compared with IPF, [†] $p < 0.05$ compared with CIP, [¶] $p < 0.05$ compared with Control diseases,

[‡] $p < 0.01$ compared with Control diseases.

**Figure 1** Serum IgG antibodies against pigeons

For definition of abbreviations, see Table 1.

* $p < 0.05$, [‡] $p < 0.01$.

On general medical examinations, there were no significant differences among the disease groups in C-reactive protein, leukocyte count, lactate dehydrogenase and the results of blood gas analysis, whereas KL-6 and SPD levels were significantly elevated in subacute HP and chronic HP. High levels of KL-6 and SPD were considered to suggest the possible diagnosis of HP. Chest HRCT findings, such as centrilobular fine granular opacity, ground-glass opacity, intraseptal thickening, traction bronchiectasis, intralobular reticular opacity, and honeycombing, pathological findings, and

BALF findings are considered useful for differentiating chronic HP from other types of IP. In many cases, however, differential diagnosis of IPF is difficult.

To identify potential causative antigens in HP, proof of specific antibodies and positive results for antigen-induced lymphocyte proliferation, environmental exposure, and antigen provocation are considered immunologically important, but these specialized tests are often not possible in general practice. Therefore, it is frequently difficult to conclusively determine the causative antigen. Some cases of chronic HP have been

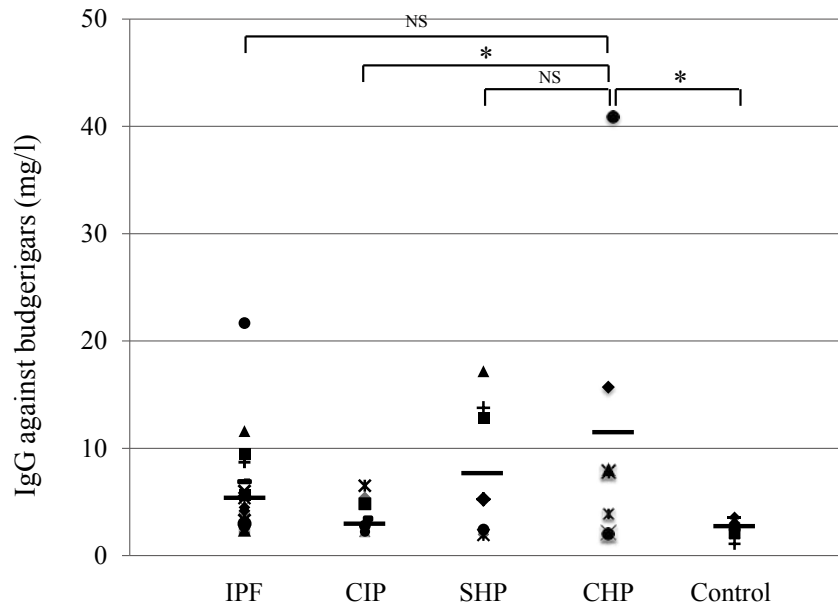


Figure 2 Serum IgG antibodies against budgerigars

For definition of abbreviations, see Table 1.

* $p < 0.05$

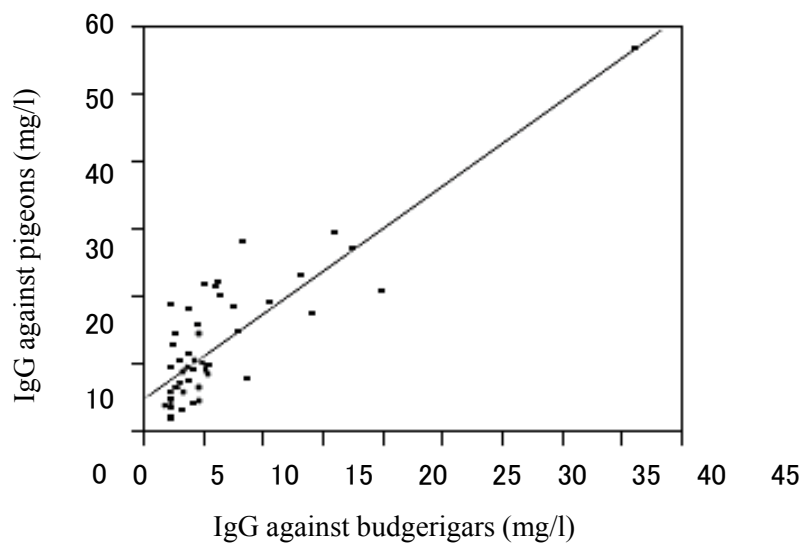


Figure 3 Correlation between IgG antibodies against pigeons and IgG antibodies against budgerigars in all subjects

$R^2 = 0.715$, $p < 0.001$.

recognized to be caused by bird-related antigens (bird waste products such as feathers or intestinal mucin contained in discharges). Potential causes of chronic BRHP include pigeons breeding in the neighborhood¹¹⁾, use of feather duvets¹²⁾, and pigeons gathering in nearby parks¹³⁾. A past history of bird breeding has been reported to be a more important factor than current bird breeding³⁾. Careful review of the patient's history

of contact with birds thus has an important role in the diagnosis of chronic HP. However, a history of contact with birds might be overlooked, especially in patients with chronic disease.

Components of the excreta of various birds may cross-react as antigens^{14~17)}. In this study, there was a strong positive correlation between anti-pigeon IgG antibody levels and anti-budgerigar IgG antibody lev-

els, suggesting that a suspected diagnosis of BRHP may only require the measurement of anti-pigeon IgG antibody. Among the different disease groups studied, anti-pigeon IgG antibody levels and anti-budgerigar IgG antibody levels were significantly elevated in patients with chronic HP. Some cases of chronic BRHP might be included among patients with a clinical diagnosis of chronic HP.

Levels of anti-pigeon IgG and anti-budgerigar IgG antibodies should be measured in healthy subjects with a history of contact with birds, bird breeding, and feather comforter use. We measured IgG antibody in 10 subjects with control diseases (lung cancer, pneumonia, and COPD). The mean anti-pigeon IgG antibody level was 8.66 ± 3.15 mg/l, and the anti-budgerigar IgG antibody level was 3.77 ± 1.05 mg/l. In a previous study, the mean anti-pigeon IgG antibody level in 42 healthy individuals was 6.64 mg/l, and the cutoff value was less than 20 mg/l¹⁸. Another study reported that the cutoff level of anti-pigeon IgG antibodies was 9.8 mg/l in 73 healthy individuals⁵. Consistent with our results, the anti-pigeon IgG antibody level in healthy subjects was not considered high.

Although IgG is usually measured as a specific antibody in HP, IgA can also be positive in this disease¹⁹. In general, IgA plays an important role in airway immunity. Antigen-specific IgA is measured in serum or in BALF in HP^{19,20}. In BRHP, the sensitivity and specificity of the IgG antibody level are equal to or higher than those of the IgA antibody level. Consequently, the value of additionally measuring IgA is considered minimal²¹.

Among the patients with a clinical diagnosis of IPF, 3 (14%) had an anti-pigeon IgG antibody level higher than 20 mg/l. The past history of contact with birds was uncertain among these patients, and some of those with a clinical diagnosis of IPF might have had chronic BRHP. In the future, anti-pigeon IgG antibody or anti-budgerigar IgG antibody levels should be measured, and detailed interviews should be conducted to ascertain the history of contact with birds in patients who are tentatively given a diagnosis IP of unknown etiology. Elevated levels of these antibodies suggest the possibility of BRHP, especially in patients with IP who have a history of contact with birds.

Our study had an important limitation. We could not

have confirmed immunological antigen provocation tests in patients with elevated levels of serum bird-specific IgG antibodies. The final definitive diagnosis of BRHP in our patients was still uncertain. If possible, it is useful to perform examinations such as pigeon serum-induced lymphocyte proliferation tests, antigen inhalation provocation tests of pigeon fecal extracts, and environmental exposure tests. The positive results of such tests will most likely contribute to the identification of etiologic agents and to diagnose BRHP.

In conclusion, high levels of anti-pigeon IgG antibodies or anti-budgerigar IgG antibodies were demonstrated using an ImmunoCAP analysis system in patients with a clinical diagnosis of chronic HP. In general, HP, especially chronic type, is difficult to definitively diagnose, and this analysis system is expected to increase the accuracy of diagnosis. Avoidance of bird-related antigens might improve the outcomes of some cases of chronic HP.

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