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ORIGINAL ARTICLE

Bronchial inflammation in hypersensitivity pneumonitis after antigen-specific inhalation challenge

ANA VILLAR,[1,2] XAVIER MUÑOZ,[1,2,3] SARA SANCHEZ-VIDAURRE,[1,2] SUSANA GÓMEZ-OLLÉS,[1,2] FERRAN MORELL[1,2] AND MARÍA-JESÚS CRUZ[1,2]

[1]*Department of Medicine, Pulmonology Unit, Vall d'Hebron University Hospital, and* [3]*Department of Cell Biology, Physiology and Immunology, Autonomous University of Barcelona, and* [2]*Network for Biomedical Research in Respiratory Disease (CIBER Enfermedades Respiratorias or CIBERES), Salud Carlos III Health Institute, Barcelona, Spain*

ABSTRACT

Background and objective: **The aim of this study is to compare the inflammatory profile before and after specific inhalation challenge (SIC) in induced sputum from patients with hypersensitivity pneumonitis (HP) and to investigate whether different causal antigens define the resulting profile.**

Methods: **A prospective study was conducted in 27 patients with HP: 15 patients due to exposure to birds (BHP) and 12 due to exposure to fungi (FHP), confirmed by SIC. Induced sputum was obtained before and/or 24 h after SIC. Cell types were determined by differential cell count using optical microscopy. Interferon-γ, interleukin (IL)-12p70, IL-2, IL-10, IL-8, IL-6, IL-4, IL-5, IL-1β, tumour necrosis factor (TNF)-α and TNF-β levels were measured in the supernatants.**

Results: **Following SIC, higher sputum neutrophilia levels ($P = 0.048$) and an increase in IL-8 levels ($P = 0.017$) were found in patients with FHP than in those with BHP. FHP patients also showed increased IL-1β, IL12-p70 and IL5 levels ($P = 0.011$, $P = 0.036$ and $P = 0.018$, respectively) after SIC. In BHP, a trend towards increases in sputum eosinophils and TH2 cytokines (IL4, IL5) was seen following SIC ($P = 0.059$, $P = 0.068$ and $P = 0.075$ respectively).**

Conclusions: **This study shows that bronchial inflammation is present in patients with HP evidenced by increases in sputum neutrophils and eosinophils following exposure to the offending antigen during SIC.**

Key words: bird, cytokine, fungus, induced sputum, neutrophil.

Correspondence: Xavier Muñoz, Servei de Pneumologia, Hospital General Vall d'Hebron, Passeig Vall d'Hebron, 119, 08035 Barcelona, Spain. Email: xmunoz@vhebron.net

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SUMMARY AT A GLANCE

Induced sputum analysis is of established value in the diagnosis and management of asthma and chronic obstructive pulmonary disease; its usefulness in interstitial lung diseases is less clear. This study shows that bronchial inflammation is present in patients with hypersensitivity pneumonitis following exposure to the offending antigen.

Abbreviations: BAL, bronchoalveolar lavage; BHP, hypersensitivity pneumonitis due to birds; DL_{CO}, diffusing capacity of the lung for carbon monoxide; FHP, hypersensitivity pneumonitis due to fungi; HP, hypersensitivity pneumonitis; IL, interleukin; SIC, specific inhalation challenge; TNF, tumour necrosis factor; VD, variable 'difference'.

INTRODUCTION

The term hypersensitivity pneumonitis (HP) refers to a heterogeneous group of diseases resulting from an inflammatory lung reaction of immunological cause in response to a wide variety of antigens, provoking varying degrees of disruption of the lung architecture.[1] In addition to a compatible clinical history, the diagnosis of HP requires determination of specific IgG antibodies,[2] consistent radiological features and lung function findings,[3] and demonstration of a lymphocytic inflammatory infiltrate in bronchoalveolar lavage (BAL) material and in tissue specimens obtained by transbronchial or surgical lung biopsy.[1] The specific inhalation challenge (SIC) can be useful for diagnosing HP,[3] although it is not necessary to establish a diagnosis.[1,4]

Induced sputum is of established value in the diagnosis and management of asthma and chronic obstructive pulmonary disease[5]; its usefulness in interstitial lung disease is less clear.[6–8] In patients with HP, the three studies with induced sputum have reported an increase in the total cell count and the lymphocyte count compared with the healthy

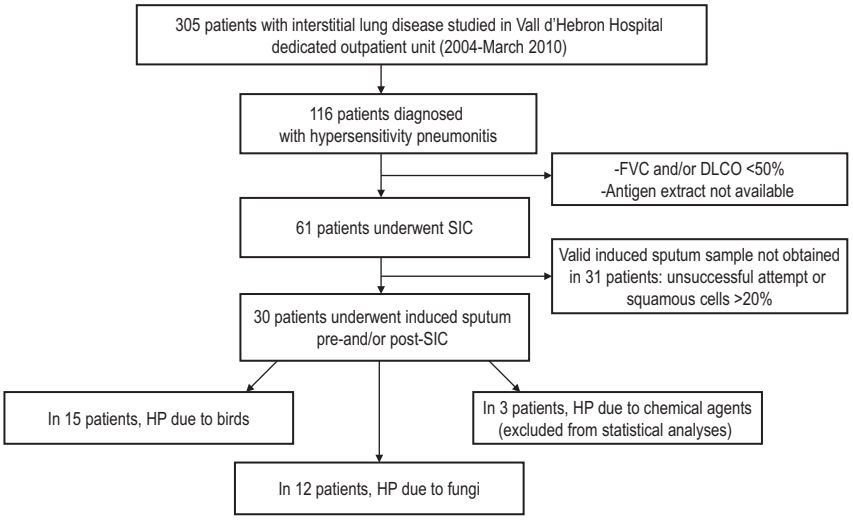


Figure 1 Flow chart of patient enrolment.

population.[6,9,10] The aim of the present study is to compare the inflammatory profile before and after SIC in induced sputum samples from patients with HP and to determine whether different causal antigens define the resulting profile.

METHODS

Study population

We studied 27 patients with HP. SIC was performed and induced sputum samples obtained before and/or after SIC (Fig. 1). Sputum samples were obtained before and after SIC in 20; before SIC, only in four, and after SIC, only in three participants. The local Ethics Committee approved the study (Hospital Vall d'Hebron Ethics Committee approval PR(AG)19/2005), and all subjects signed an informed consent.

Diagnostic criteria

The diagnosis of HP was established according to the criteria proposed by Schuyler and Cormier[4] (Table 1). The form of presentation was considered acute when the symptoms (general malaise, dyspnea, cough with/without expectoration, fever, chest pain) appeared a few hours after exposure to the offending antigen and disappeared when the causal agent was avoided. The subacute form was established when symptoms such as asthenia, constitutional syndrome, low-grade fever, weight loss, dry and/or productive cough, or dyspnea on exercise developed over a period of weeks or months. Patients were considered to have the chronic form when they presented with grade III/IV dyspnea according to the New York Heart Association or Medical Research Council scale with or without current or previous antigen exposure, and computed tomography or lung biopsy demonstrated diffuse interstitial lung disease with established fibrosis.[3]

The intensity of exposure was quantified based on its total duration; that is, according to the total of hours the patient was exposed to the antigenic source, calculated from the data obtained in his/her clinical records. The modified criteria of Rodríguez de Castro *et al.*[11] were used to classify exposure into three categories: *mild* (including sporadic exposure and exposure of short duration), 1–6 h per week; *moderate*, 6–18 h per week; and *intense*, more than 18 h per week.

Table 1 Major and minor criteria for the diagnosis of hypersensitivity pneumonitis. Patients had to present at least four major criteria and two minor criteria; other conditions with similar characteristics were ruled out

Major criteria:
Symptoms consistent with HP.
Evidence of appropriate antigen exposure in medical history and/or detection of specific precipitins in serum and/or BAL findings consistent with HP on chest plain films or chest CT.
Lymphocytosis in BAL (when performed).
Histological changes consistent with HP.
Positive SIC (reproduction of symptoms and laboratory abnormalities following exposure to the suspected antigen).
Minor criteria:
Bilateral basal crackles.
Decrease in DL_{CO}.
Arterial hypoxemia, at least following exercise.

BAL, bronchoalveolar lavage; DL_{CO}, diffusing capacity of the lung for carbon monoxide; HP, hypersensitivity pneumonitis; SIC, specific inhalation challenge.

Lung function studies

All patients underwent spirometry, lung volume by plethysmography and determination of the diffusing capacity of the lung for carbon monoxide (DL_{CO}) using the single breath-hold method. These studies were performed on a MasterLab system (MasterLab, Jaeger, Germany) following the joint recommendations of the European Respiratory Society and American Thoracic Society.[12] The forced spirometry reference

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values were those proposed for the Mediterranean population.[13] For the diffusion study, the single-breath DL_{CO} was used. The theoretical values were those proposed by the European Respiratory Society.[14]

Antigen extract preparation for SIC

Commercialized extracts (Bial-Aristegui, Bilbao, Spain) from *Penicillium frequentans*, *Aspergillus fumigatus* and *Mucor mucedo* were used to study fungi.[15] The avian sera and pigeon bloom extracts were prepared in our laboratory as previously described.[3,15]

SIC

A de Vilbiss 646 nebulizer (de Vilbiss Health Care LLC, Somerset, PA, USA) and Mefar MB3 dosimeter (Mefar srl, Bovezzi, Italy) were used, which release the antigenic solution during the first second of each inhalation. The technique consisted of inhaling 2 mL of solution at a 1/100 (0.01 mg/mL) dilution. The patients' forced vital capacity (FVC), forced expiratory volume in 1 s, DL_{CO} and temperature were assessed at baseline, at 20 min following exposure and every hour thereafter for the next 8 h. The SIC was considered positive according to previously published criteria[3,15] (Table 2). In patients testing negative on SIC, exposure was repeated 24 h later, at an antigen dilution of 1/10 (0.1 mg/mL). In all cases, a baseline test was performed with placebo solution the day before inhalation of the putative causal agent.

The SIC was performed to pigeon serum ($n = 7$), parakeet serum ($n = 5$), goldfinch serum ($n = 2$), goose feathers ($n = 1$), *P. frequentans* extract ($n = 8$), *A. fumigatus* extract ($n = 2$) and *M. mucedo* extract ($n = 2$).

Induced sputum

Sputum induction was performed using the method described by Pizzichini *et al.*[16] An aerosol of hypertonic saline generated by an OMRON ultrasonic nebulizer (Peróxidos Famacéuticos S.A., Barcelona, Spain) was inhaled through a mouthpiece with a nose clip in place at increasing concentrations of 3%, 4% and 5% for 7 min per concentration. All sputum samples were collected the day prior to the completion of the SIC and the day after the last exposure. In all patients, the time interval between SIC and sputum collection was the same.

Sputum samples were processed within 2 h, as described by Pizzichini *et al.*[16] Total cell count, cell viability and percentage of squamous cells were determined using trypan blue exclusion staining in a Neubauer hematocytometer. Differential cell counts of macrophages, neutrophils, lymphocytes and eosinophils were carried out by optical microscopy and expressed as percentages of the total of non-squamous cells.

Cytokine measurement

Interleukin (IL)12-p70, interferon-γ, IL-2, IL-10, IL-8, IL-6, IL-4, IL-5, IL-1β, tumour necrosis factor (TNF)-α and TNF-β levels in sputum sample supernatants were determined by the FlowCytomix bead-based assay (Bender Medsystems, GmbH, Burlingame, CA, USA) which applies the same principle as a sandwich immunoassay. The limits of sensitivity (pg/mL) were 1.5, 1.6, 16.4, 1.9, 0.5, 1.2, 20.8, 1.6, 4.2, 3.2 and 2.4 respectively.

Statistical analysis

Results were expressed as the median and range unless otherwise stated. Between-group and within-group differences were analysed using the Mann–Whitney test and Wilcoxon signed rank test respectively. In induced sputum, baseline was calculated as percentage cell type, and differences were calculated as percentage change from baseline. The variable 'difference' (VD) for differential cell count and cytokines was calculated by subtracting the value before SIC from the value after SIC. SPSS 17.0 for Windows (SPSS, INC, Chicago, IL, USA) was used for the statistical analyses.

Table 2 Criteria for SIC positivity

The test is considered positive when any of the following responses is elicited:

(1) FVC decrease >15% or DL_{CO} decrease >20% as compared with baseline values.
(2) 10–15% FVC decrease plus at least one of the following criteria with respect to clinical status and basal analytic values:
  (a) White blood cell increase ≥20%
  (b) O_2 saturation decrease ≥3%
  (c) Significant radiologic changes
  (d) Rise in body temperature >0.5°C
  (e) Clinical symptoms (e.g. cough, dyspnea)
(3) FVC decrease <10% but with evidence of three or more of the previously mentioned clinical or analytic criteria.

DL_{CO}, carbon monoxide diffusing capacity; FVC, forced vital capacity; SIC, specific inhalation challenge.

RESULTS

Patients' demographic data and clinical characteristics are shown in Table 3. Chest tightness was more frequent in patients with hypersensitivity pneumonitis due to birds (BHP) than in patients with hypersensitivity pneumonitis due to fungi (FHP) ($P = 0.034$). The results obtained in the ancillary examinations performed are shown in Table 4. Regardless of diagnosis, BAL was performed in 23 patients, and in 11 (47.8%), a BAL lymphocytosis (a cellular count higher than 20%) was found. Transbronchial biopsy was performed in 15 patients, showing characteristic HP pattern in one patient, non-specific interstitial pneumonia pattern in another patient and unspecific fibrosis in the remaining patients. Finally surgical lung biopsy was performed in five patients, finding an HP pattern in two patients, a non-specific interstitial pneumonia pattern in one patient and

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Table 3 Clinical data and demographic characteristics of the study population

	Total *n* = 27	BHP *n* = 15	FHP *n* = 12	*P**
Gender, male/female	7/20	4/11	3/9	0.636
Age, median (range)	57 (26–80)	57 (26–76)	60 (31–80)	0.683
Smoking habit, *n* (%)				0.782
Smoker	4 (15%)	2 (13%)	2 (17%)	
Non-smoker	11 (41%)	7 (48%)	4 (33%)	
Ex-smoker	12 (44%)	6 (40%)	6 (50%)	
Packet-years**	10 (0.40)	12 (2–40)	10 (0–35)	0.950
Symptoms, *n* (%)				
Fever	9 (41%)	5 (33%)	6 (50%)	0.315
Cough	21 (78%)	11 (73%)	10(83%)	0.443
Dyspnea	26 (96%)	14 (93%)	12(100%)	0.556
Chest tightness	18 (69%)	13 (86%)	5 (45%)	0.034
Joint pain	17 (63%)	12 (86%)	6 (50%)	0.204
Auscultation, *n* (%)				0.401
Normal	14 (52%)	10 (67%)	4 (33%)	
Crackles	9 (33%)	4 (27%)	5 (42%)	
Wheezing	4 (15%)	1 (6%)	3 (25%)	
Total exp time, month	156 (3–552)	152 (36–552)	162 (3–492)	0.605
Latency period, month	140 (1–720)	149 (5–720)	126 (1–480)	0.935
Exp-sympt time, month	40 (3–240)	33 (3–84)	51 (4–240)	0.849
Intensity exp, *n* (%)				0.686
Mild	7 (26%)	5 (33%)	2 (17%)	
Moderate	4 (15%)	2 (13%)	2 (17%)	
Intense	16 (59%)	8 (54%)	8 (66%)	
Clinical presentation, *n* (%)				0.707
Acute	3 (11%)	2 (13%)	1 (8%)	
Subacute	13 (48%)	8 (53%)	5 (42%)	
Chronic	11 (41%)	5 (34%)	6 (50%)	

Data expressed as median and range unless otherwise stated.
* Birds versus fungi; ** Includes smokers and ex-smokers.
Total exp time, time from start of exposure to diagnosis.
Latency period, time from start of exposure to start of symptoms.
Exp-sympt time, time from start of symptoms to diagnosis.

centrilobularfibrosis in two patients. The SIC was considered positive according to previously published criteria.[3,15] The test was positive in all of the 27 patients included in the study. After SIC, a decrease in FVC and/or DL_{CO} greater than 15% and/or than 20% respectively was observed in 17 patients. In five patients, a 10–15% decline in FVC and at least one of the defined criteria[3,15] was observed; in five patients, the decrease of the FVC was less than 10% with all defining criteria observed.

The results of sputum sample before and after SIC are shown in Table 5 and in Figure 2. There were no significant differences between the baseline characteristics of the HP patients included in the study and the ones who could not produce sputum. Pre- and post-SIC neutrophil levels were higher in both types of HP than the expected values in the healthy population.[19] Neutrophil levels rose further in the FHP group following SIC, although the difference only presented a trend ($P = 0.059$). This finding was not observed in patients with BHP. Post-SIC neutrophil percentage was higher when the causal agents were fungi ($P = 0.048$). An increase in eosinophils following SIC was noted in the total population ($P = 0.013$). In the analysis according to the causal agent, the eosinophil increase was only observed in BHP patients, although the result was again not statistically significant ($P = 0.059$). Analysis of the VD of sputum neutrophils showed higher values in the FHP group than in the BHP group, although the result was not statistically significant ($P = 0.063$) (Table 5). We found no correlation between the percentages of neutrophils and eosinophils in BAL and sputum in the two groups.

Cytokines measurements in induced sputum before and after SIC are shown in Table 6 and Figure 3. Post-SIC IL8 and TNFα levels were higher in FHP compared with BHP ($P = 0.017$ and $P = 0.035$ respectively). An increase in proinflammatory cytokines—IL12-p70 ($P = 0.036$) and IL1β ($P = 0.011$)—and in IL5 ($P = 0.018$) was recorded in FHP patients following SIC. In patients with BHP, a trend towards increased Th2 cytokines (IL4, IL5) was observed, though without reaching statistical significance. Analysis of the VD of cytokines measurement showed higher values of IL12-p70 and IL5 in the FHP group

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Table 4 Ancillary tests

	Total $n = 27$	BHP $n = 15$	FHP $n = 12$	P*
Peripheral blood				
Leukocytes/mm^3	7900	7800	8100	0.300
Cell count (%)				
Neutrophils	61.0 (37–85)	60.0 (37.0–68.0)	66.0 (40.0–85.0)	0.114
Lymphocytes	28.4 (11.0–46.0)	31.5 (17.0–43.0)	23.0 (11.0–46.0)	0.167
Monocytes	6.9 (3.0–13.4)	8.1 (5.3–13.4)	6.6 (3.0–10.0)	
Eosinophils	2.0 (0.0–12.0)	1.9 (0.0–8.4)	2.3 (0.7–12.0)	
Radiologic pattern**, *n* (%)				0.727
Normal	4 (15%)	2 (13%)	2 (17%)	
Reticular	1 (4%)	–	1 (8%)	
Nodular	8 (29%)	5 (33%)	3 (25%)	
UIP	2 (7%)	1 (7%)	1 (8%)	
Ground glass	11 (41%)	7 (47%)	4 (34%)	
OP	1 (4%)	–	1 (8%)	
Pulmonary function test				
FVC (%predicted)	76 (43–113)	73 (43–113)	77 (60–102)	0.486
FEV_1 (%predicted)	84 (43–124)	87 (43–124)	84 (61–110)	0.755
FEV_1/FVC%	82 (55–92)	81 (73–92)	83 (55–88)	0.905
DL_{CO} (%predicted)	60 (35–95)	58 (35–92)	60.5 (36–95)	0.829
KCO (%predicted)	75 (39–105)	75 (39–105)	74 (48–101)	0.648
Bronchoalveolar lavage, %				
Neutrophils	10 (0–62)	10 (2–18)	5 (0–62)	0.948
Lymphocytes	13 (2–86)	10 (2–86)	17 (10–40)	0.250
Macrophages	74 (14–89)	80 (14–89)	64 (22–85)	0.164
Eosinophils	0.46 (0–14)	0 (0–1)	0 (0–5)	
Specific IgG, A_{450} nm				0.077
Positive	16/23 (70%)	7/13 (54%)	9/10 (90%)	
Immediate hypersensitivity skin test***				0.535
Positive	16/20 (80%)	10/12 (83%)	6/8 (75%)	

Data expressed as median and range.

* Birds versus fungi.

** See references, [17,18].

*** Pigeon bloom, and pigeon, parakeet, canary, hen, parrot sera, *Aspergillus fumigatus, Penicillium frequentans, Mucor mucedo* and/or *Rhizopus nigricans*.

A_{450} nm, absorbance units at 450 nm; BHP, hypersensitivity pneumonitis due to birds; DL_{CO}, carbon monoxide diffusing capacity; FEV_1, forced expiratory volume in 1 s; FHP, hypersensitivity pneumonitis due to fungi; FVC, forced vital capacity; KCO, transfer factor; OP, organizing pneumonia; UIP, usual interstitial pneumonia.

compared with BHP group, although for IL-5, the result was not statistically significant ($P = 0.033$ and 0.081, respectively) (Table 6).

DISCUSSION

This study is the first to analyse the induced sputum inflammatory profile in two groups of patients with HP secondary to different antigens. The results show a similar baseline neutrophilic pattern in HP caused by contact with fungi and with birds. Following exposure patients with FHP presented increases in neutrophils and proinflammatory cytokines, whereas those with BHP showed increases in eosinophils and Th2 cytokines.

These results prompt the hypothesis that different antigens may cause HP to develop by different mechanisms. Following Pepys *et al.'s*[20] definition of HP and the finding of specific IgG antibodies in most HP patients, a type III hypersensitivity mechanism was thought to be the causal mechanism. Currently, it is believed that the mechanism responsible may be a T-cell-mediated type IV hypersensitivity response.[21] Several authors have suggested that in patients displaying acute or subacute symptoms, a Th1 mechanism may be involved, while in those progressing to pulmonary fibrosis, a Th2 mechanism could be responsible for the disease.[22,23] Doubts have arisen whether the degree of exposure leads to the immunologic response rather than the antigen itself. It is uncertain whether individual susceptibility could be the factor (independently of the antigen and degree of exposure) that determines the specific immunologic response and/or the form of presentation. Some authors have suggested that the immunological mechanism involved determines both the type of antigen and form of presentation, based on a predominance of CD4 or CD8 cells in different types of HP.[24] In our setting, the most common types of HP are

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Table 5 Results of induced sputum cell analysis

	Total	BHP	FHP	$p^{¶}$
Before SIC	$n = 24$	$n = 13$	$n = 11$	
Total cell count ($\times 10^6$)	0.46 (0.04–14)	0.19 (1.1–1.6)	0.5 (0.04–13.8)	0.566
Differential cell count (%)				
Neutrophils	78 (28–92)	64 (40–90)	82 (28–92)	0.331
Lymphocytes	0 (0–4)	1 (0–4)	0 (0–1)	0.189
Macrophages	20 (8–71)	35 (9–52)	17 (8–71)	0.270
Eosinophils	1 (0–4)	1 (0–4)	1 (0–3)	0.975
After SIC	$n = 23$	$n = 11$	$n = 12$	
Total cell count ($\times 10^6$)	0.93 (0.1–9)	0.63 (0.1–5.1)	1.54 (0.14–9.1)	0.235
Differential cell count (%)				
Neutrophils	81 (45–94)	81 (45–90)	87.5 (58–94)***	0.048
Lymphocytes	1 (0–4)	1 (0–4)	0.5 (0–1)	0.559
Macrophages	17 (5–53)	19 (10–53)	11.5 (5–38)$^{+}$	0.021
Eosinophils	1 (0–7)*	1 (0–6)**	1 (0–7)	0.657
Difference	$n = 23$	$n = 11$	$n = 11$	
Total cell count ($\times 10^6$)	0.18 (−4.76–3.36)	0.17 (−0.98–3.36)	0.22 (−4.76–2.44)	0.968
Differential cell count (%)				
Neutrophils	7 (−32–30)	−5 (−32–30)	10 (−8–30)	0.063
Lymphocytes	0 (−3–1)	0 (−3–1)	0 (0–1)	0.340
Macrophages	−7 (−33–32)	5 (−28–32)	−10 (−33–6)	0.053
Eosinophils	0 (−1–5)	0 (0–2)	0 (−1–5)	0.842

Data expressed as the median (range). Variable 'difference' calculated by subtracting the value before SIC from the value after SIC.
¶ birds versus fungi.
Comparison before and after SIC (calculated as a percentage change from baseline): * $P = 0.013$; ** $P = 0.059$; *** $P = 0.059$; $^{+}P = 0.041$.
BHP, hypersensitivity pneumonitis due to birds; FHP, hypersensitivity pneumonitis due to fungi; SIC, specific inhalation challenge.

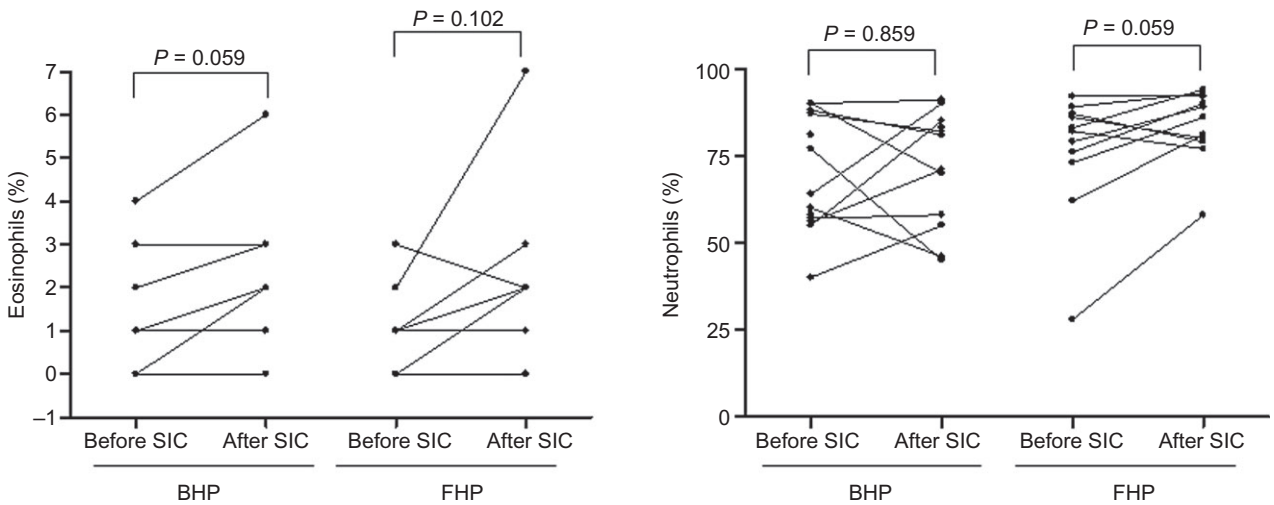


Figure 2 Differential cell count in sputum samples.

bird fancier's lung,[3] farmer's lung,[25] espartosis (pneumonitis due to esparto grass) in construction workers[26] and suberosis in cork workers.[15] The causal agents implicated in these last three conditions are all fungi, and the diseases are characterized by work-related exposure: duration is longer and intensity of exposure greater than in the case of other HPs such as contact with avian antigens, in which exposure is generally intermittent and low intensity.[3] This differential feature could explain the development of distinct inflammatory responses, that is, FHP involves a higher degree of inflammation than BHP, following exposure to the causal agent.

HP is characterized by T cell-dependent alveolitis. Few studies have analysed the type of bronchial or bronchiolar inflammation. Sobiecka *et al.*[6] described an increase in neutrophils and a decrease in lymphocytes in induced sputum relative to BAL. D'Ippolito

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Table 6 Inflammatory cytokines analysis in induced sputum

	Total	BHP	FHP	$p^{¶}$
Before SIC	*n* = 24	*n* = 13	*n* = 11	
IL12-p70	10.74 (1–21)	11.97 (1–17)	10.04 (1–24)	0.391
IFN-gamma	21.48 (2–36)	21.48 (2–28)	21.48 (2–36)	0.336
IL-2	36.82 (16–122)	16.4 (16–101)	39.55 (16–122)	0.324
IL-10	12.02 (2–53)	8.53 (3–53)	12.66 (2–34)	0.490
IL-8	4 656.63 (367–13 356)	4 253.08 (366–9 384)	6 859.56 (1 011–13 356)	0.140
IL-6	29.54 (8–433)	22.61 (7–432)	32.06 (16–326)	0.532
IL-4	20.80 (21–100)	20.80 (20–22)	20.80 (20–99)	0.098
IL-5	41.08 (2–621)	43.88 (2–543)	38.28 (2–621)	0.595
IL-1-beta	48.46 (4–2 110)	59.11 (4–631)	37.82 (4–2 110)	0.683
TNF-alpha	4.76 (3–97)	3.61 (3–97)	7.36 (3–58)	0.189
TNF-beta	2.4 (2–18)	2.40 (2–6)	2.40 (2–18)	0.475
After SIC	*n* = 23	*n* = 11	*n* = 12	
IL12-p70	10.76 (1–28)	9.03 (1–18)	12.70 (1–28)†	0.382
IFN-gamma	22.95 (2–61)	23.05 (2–31)	22.25 (2–61)	0.509
IL-2	61.37 (14–232)	53.76 (14–179)	62.12 (16–232)	0.619
IL-10	15.02 (3–60)	10.97 (4–25)	20.53 (3–60)	0.382
IL-8	5 929.41 (458–12 181)	4 461 (458–10 048)	8 846.85 (2 461–12 180)	0.017
IL-6	65.81 (9–747)	63.02 (9–197)	89.30 (19–747)	0.305
IL-4	20.80 (10–178)	20.8 (20–57)*	20.80 (10–178)	0.391
IL-5	63.88 (2–737)	49.53 (2–543)**	185.97 (2–736)††	0.253
IL-1-beta	101.19 (4–3 252)	10.99 (4–862)	195.79 (4–3 252)†††	0.090
TNF-alpha	6.88 (3–124)	5.46 (3–15)	15.45 (3–124)	0.035
TNF-beta	2.4 (2–57)	2.40 (2–17)	2.40 (2–57)	0.664
Difference	*n* = 23	*n* = 11	*n* = 11	
IL12-p70	0.72 (−10.47–18.52)	−0.79 (−10.47–8.76)	3.39 (−1.75–18.52)	0.033
IFN-gamma	0 (−18.93–25.89)	0 (−18.74–21.25)	0.77 (−18.93–25.89)	0.879
IL-2	14.04 (−51.52–109.62)	12.51 (−51.52–109.62)	15.58 (−18.71–109.55)	0.909
IL-10	2.61 (−36.14–25.44)	−0.72 (−36.14–9.69)	2.95 (−8.11–25.44)	0.569
IL-8	−272.82 (−4 395.77–6 307.71)	−222.45 (−4 395.77–5 250.87)	−323.19 (−3 421.38–6 307.71)	0.621
IL-6	3.74 (−235.42–527.86)	0 (−235.42–156.32)	11.54 (−227.12–527.86)	0.470
IL-4	0 (−25.79–78.44)	0 (0–36.28)	0 (−25.79–78.44)	0.072
IL-5	30.99 (−11.17–270.96)	0 (−11.17–229.86)	65.10 (0–270.96)	0.081
IL-1-beta	51.98 (−155.92–1 141.80)	0 (−155.92–650.68)	70.45 (−6.79–1 141.8)	0.158
TNF-alpha	0.23 (−83.09–65.99)	0 (−83.09–6.90)	3.20 (−2.83–65.99)	0.235
TNF-beta	0 (−15.74–54.91)	0 (0–10.99)	0 (−15.74–54.91)	0.960

Data expressed as the median and range (pg/mL). Variable 'difference' calculated by subtracting the value before SIC from the value after SIC. ¶ birds versus fungi.

Comparison before and after SIC: † $P = 0.036$; †† $P = 0.018$; ††† $P = 0.011$; * $P = 0.068$; ** $P = 0.075$.

BHP, hypersensitivity pneumonitis due to birds; FHP, hypersensitivity pneumonitis due to fungi; IFN, Interferon; IL, Interleukin; SIC, specific inhalation challenge; TNF, Tumour necrosis factor.

et al.[9] studied nine HP patients, seven secondary to *Micropolyspora faeni* and two secondary to avian antigens, with a subacute presentation in eight cases and chronic presentation in one. A marked fall in lymphocytes and an increase in neutrophils as compared with BAL were reported. In this study, the same pattern was found, although the percentage of neutrophils in induced sputum was higher than the values found by D'Ippolito *et al.* and the percentage of lymphocytes lower. This difference, particularly with regard to lymphocytes, may be due to the fact that the lymphocyte count in BAL was relatively low in our patients, with a median of 10% in BHP and 17% in FHP, probably due to the fact that the majority of the patients included in the study had a chronic form of HP.[3] We also note that in a disease mediated by lymphocytes, the predominant inflammation in the bronchi was neutrophilic, a circumstance that is not exclusive to HP. Similar features have been observed in patients with idiopathic pulmonary fibrosis, sarcoidosis and scleroderma.[6–10] This may be because BAL provides information on the distal airway and alveolar spaces, which are rich in macrophages, whereas induced sputum gives evidence of the state of the upper and middle airway, which presents abundant neutrophils.[27,28] Furthermore, in FHP patients, the significant increase in IL-8, IL12-p70 and IL-1β as compared with BHP could explain the greater increase in neutrophils observed following exposure to fungi. Lastly, another possibility would be related to the innate immune response. Recent studies have shown an essential role of CD17 lymphocytes and

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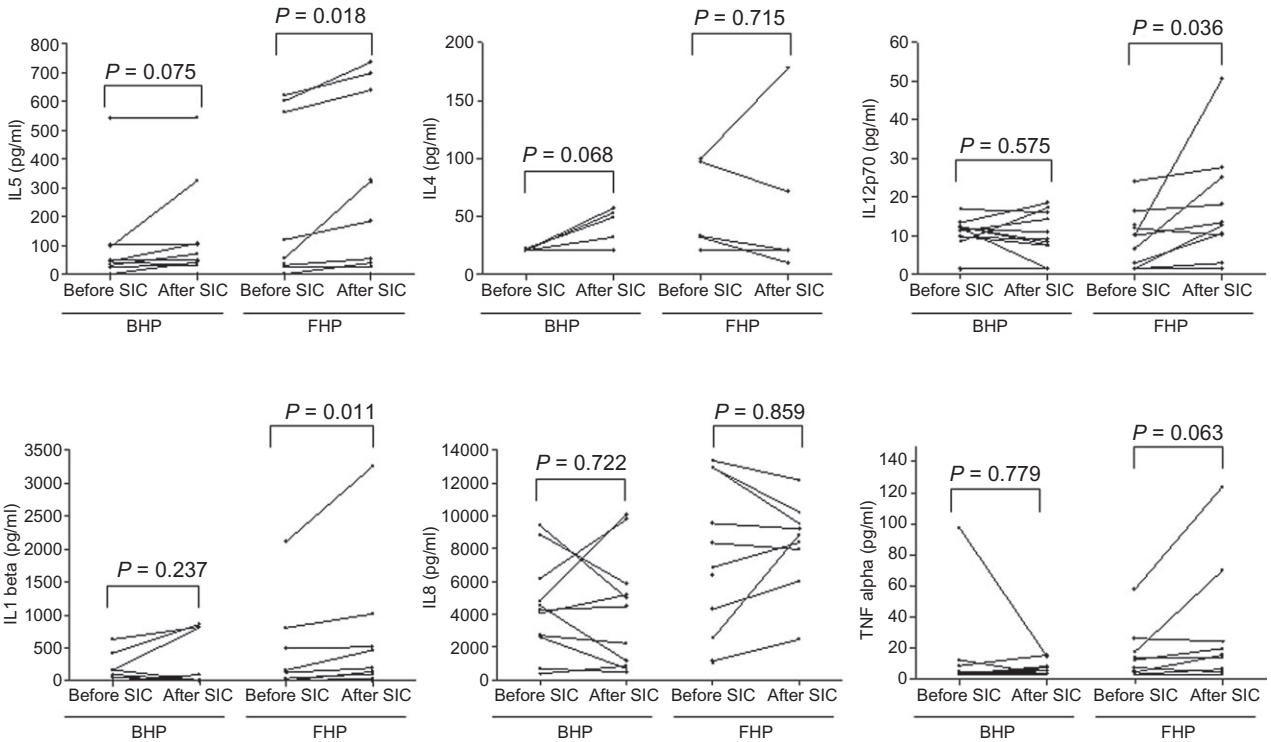


Figure 3 Cytokines in sputum supernatant.

IL-17 in the immunopathology of HP;[29] the IL-17-mediated response is basically neutrophilic. Recent investigations have shown that airway exposure to antigens in sensitized individuals causes release of IL-17, which orchestrates airway inflammation by inducing the expression of various pro-inflammatory mediators such as cytokines, chemokines and adhesion molecules, in turn leading to recruitment and activation and of neutrophils and Th2-mediated eosinophils.[30]

An interesting finding in the present study and in those of Sobiecka *et al.*[6] and D'Ippolito *et al.*[9] is the mild eosinophilic inflammation in sputum of patients with HP. Since the Th2 response is implicated in the immunopathology of HP,[22,23] the presence of eosinophilic inflammation may be no surprise. In fact, several studies have demonstrated eosinophils in BAL[31] and pulmonary biopsies.[32] The eosinophil counts and levels of IL-4 and IL-5 (Th2 cytokines) increased in patients with BHP following SIC. However, the increase of IL-5 found in patients with FHP does not rule out this response in BHP, as we found no significant differences between BHP and FHP patients in other aspects like the presentation, intensity of exposure, pulmonary function results or BAL findings.

Although this study is the first to analyse cell changes in induced sputum following antigen exposure in a relatively large number of HP patients, it is not without limitations. The most important is the lack of a control group of healthy individuals in order to determine whether inhalation of fungal or avian antigens causes bronchial inflammation. Some authors argue that inhalation of antigens during SIC may worsen the symptoms of an HP patient, and sensitize patients without this condition or with another underlying disease.[33] Although most authors argue the risk is acceptable in patients in view of the major benefits of a correct diagnosis,[3,34,35] it may not be justified in healthy individuals for scientific purposes. Another limitation is the small number of patients ultimately included. The main reason for this was that induced sputum could not be obtained in 50% of HP patients who underwent SIC. This limited yield is similar to the performance reported by other authors using this technique in patients with interstitial lung disease.[6,9,28]

In conclusion, this study shows that bronchial inflammation is present in patients with HP, as evidenced by increases in neutrophils and eosinophils following exposure to the offending antigen during SIC. Sputum neutrophilic inflammation increases after SIC with fungal antigens, with a predominance of proinflammatory cytokines, and eosinophilic inflammation is triggered after SIC with avian antigens, with a predominance of Th2 cytokines. However, the difference in neutrophilic or eosinophilic response between BHP and FHP isn't clear enough to draw firm conclusions. Future studies are needed, with larger numbers of patients, to confirm these findings.

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