Allergy 2009: 64: 322–334

DOI: 10.1111/j.1398-9995.2009.01949.x

Review article

Hypersensitivity pneumonitis

The first few cases of hypersensitivity pneumonitis (HP) were described in the early 20th century in farmers exposed to moldy hay or straw. As then, HP has been ascribed to multiple inhaled antigens found in a large variety of environmental settings. Hypersensitivity pneumonitis results from an exaggerated immune response, which gives rise to acute infection-like symptoms or to progressive, sometimes irreversible lung damage. The diagnosis is based on a combination of clinical characteristics of the disease. Clinical diagnostic criteria have recently been published. The immune mechanisms leading to HP are still incompletely understood. Initially, believed to be a classes III and IV immune response, we now have a clearer understanding of the complex inflammatory events involved. These include the release of pro inflammatory cytokines and a decrease in the immune control mechanisms via surfactant, dendritic and T-regulatory cells. Despite the improved understanding, the treatment and outcome of HP have not changed. Oral corticosteroids remain the only effective drugs and contact withdrawal constitutes the ideal solution. If unchecked, HP can lead to irreversible lung damage in the form of fibrosis or emphysema, respiratory insufficiency and even death.

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Key words: environment; immunology; inflammation; interstitial lung disease; organic dust.

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Accepted for publication 2 October 2008

Hypersensitivity pneumonitis (HP) is the term now most commonly used to describe a disease that was previously referred to as extrinsic allergic alveolitis. It is a disease that occurs upon exposure to organic dust. Initially, it was associated with farming (moldy grain or hay handling) hence the term farmer's lung (1, 2). With time, a large variety of environmental settings and antigens have been described (3). Among the other most common settings are contacts with birds (pigeons, parakeets), humidifiers, moldy wood, and a variety of other settings where moulds abound. Although most antigens are organic particles, some chemical compounds (e.g. isocyanates, zinc,) can act as haptens which link to the host albumin to create an antigenic particle.

Although HP is a well-known clinical entity, there is currently no clear and universally accepted definition. The HP study group simply defined HP as: 'a pulmonary disease with symptoms of dyspnoea and cough resulting from the inhalation of an antigen to which the patient has been previously sensitized' (4). The NHLBI/ORD workshop report does not provide any definition but address the need for precise diagnostic criteria and stress the importance of history, clinical manifestations, clinical course and exposure in establishing the diagnosis (5).

Cormier and Schuyler (3) previously suggested the following definition: 'HP is an inappropriate immune response to inhaled antigens that causes shortness of breath, a restrictive lung defect, interstitial infiltrates seen on lung imaging [chest X-ray and high-resolution computed tomography (HRCT)] caused by the accumulation of large numbers of activated T lymphocytes in the lungs. The disease is sometimes further characterized by episodic bouts of fever a few hours after exposure' (3).

The objective of this review is to provide a broad picture of the epidemiology, pathophysiology and clinical aspects of HP that still challenge researchers and clinicians (5).

Epidemiology

As is the case with most interstitial lung diseases, HP is an orphan disease. In a population-based study, the estimated annual incidence of interstitial lung diseases was 30 per 100 000 (6). In that study, HP accounted for less than 2% of the incident cases. The study was conducted in New Mexico, a dry environment that is not propitious to the development of many forms of HP.

Over the last two or three decades, the difficulties in studying the epidemiology of HP have been illustrated in studies of the incidence or prevalence of farmer's lung. Definite conclusions have been elusive because of methodological issues including study design and the definition of farmer's lung (7–9). Most studies used cross-sectional

surveys in order to determine the prevalence of farmer's lung or that of associated conditions such as the presence of precipitating antibodies against offending antigens. Few, if any, real cohort studies have been published on the incidence of the disease (10–12). An even more important factor has been the lack of a consistent definition of farmer's lung. Epidemiologic reports based on cases admitted to a hospital where a definite diagnosis can be made using chest radiographs, CT, bronchoalveolar lavage (BAL) and/or lung biopsies are likely to identify the most severe cases only and thus underestimate the true prevalence of the disease. In addition, important differences have been observed in the classification of respiratory diseases among farmers by clinicians from different European countries (13). In a survey of final diagnostic classifications on hospital discharge, 73% of cases of HP were erroneously classified (14). Finally, fluctuations in the prevalence of farmer's lung have been related to a greater diagnostic suspicion attributable to ongoing epidemiological surveys (15). The difficulties in establishing the incidence and prevalence of HP are further complicated by geographical variables, including climatic conditions and, in the case of farmer's lung, farming practices. Gender differences for both HP and seropositivity are likely to represent differences in exposures to offending antigens (16–18). Despite these methodological limitations, several studies gave consistent results allowing the estimation of the prevalence of farmer's lung in exposed farmers from 0.5% to 3% (19–24).

Diagnostic criteria

A number of diagnostic criteria recommendations for HP have been published (12, 25–27) (Table 1). The most widely used are those from Richerson et al. (25). None of these sets of criteria have been validated. Their diagnostic accuracy is therefore unknown. They correspond in effect to definitions of the disease.

Others have developed prediction rules [i.e. clinical tools that quantify the contribution of various components of the history, physical examination and basic laboratory results in the diagnosis in an individual patient (28)] for periodic surveillance in high-risk workers or case finding in outbreaks of HP (29–31). Although these rules are meant to be sensitive (i.e. able to detect most cases of work-related HP), it is likely that their specificity is limited in work environments with a high prevalence of other respiratory diseases. Little information is provided concerning their accuracy.

Table 1. Proposed diagnostic criteria for hypersensitivity pneumonitis for clinical purposes

Author	Major criteria	Minor criteria
Terho (12)	1. Exposure to offending antigens (revealed by history aerobiological or microbiologic investigations of the environment, or measurements of antigen-specific IgG antibodies 2. Symptoms compatible with HP present and appearing or worsening some hours after antigen exposure 3. Lung infiltrations compatible with HP visible on chest X-ray	1. Basal crepitant rales 2. Impairment of the diffusing capacity 3. Oxygen tension (or saturation) of the arterial blood either decreased at rest, or normal at rest but decreased during exercise 4. Restrictive ventilation defect in the spirometry 5. Histological changes compatible with HP 6. Positive provocation test whether by work exposure or by controlled inhalation challenge
Richerson et al. (25)	1. The history and physical findings and pulmonary function tests indicate an interstitial lung disease 2. The X-ray film is consistent 3. There is exposure to a recognized cause 4. There is antibody to that antigen	
Cormier et al. (126)	1. Appropriate exposure 2. Inspiratory crackles 3. Lymphocytic alveolitis (if BAL is done) 4. Dyspnoea 5. Infiltrates on chest radiographs (or HRCT)	1. Recurrent febrile episodes 2. Decreased DLCO 3. Precipitating antibodies to HP antigens 4. Granulomas on lung biopsy (usually not required) 5. Improvement with contact avoidance or appropriate treatment
Schuyler (27)	1. Symptoms compatible with HP 2. Evidence of exposure to appropriate antigen by history or detection in serum and/or BAL fluid antibody 3. Findings compatible with HP on chest radiograph or HRCT 4. BAL fluid lymphocytosis 5. Pulmonary histological changes compatible with HP 6. Positive 'natural challenge'	1. Bibasilar rales 2. Decreased DLCO 3. Arterial hypoxaemia, either at rest or during exercise

The HP study

We addressed the issue of the clinical diagnostic criteria of HP in a prospective multi-centre cohort study (4). Its objective was to develop a clinical prediction rule for the diagnosis of active HP. Such a rule aims at helping clinicians to arrive at a more accurate estimate of probability of HP and decide whether further investigation is needed to either rule in or rule out HP.

Consecutive adult patients presenting with a pulmonary syndrome for which active HP was considered in the differential diagnosis were included in this study. This cohort thus included a wide range of patients presenting for the investigation of a suspected interstitial lung disease, including patients with HP (the 'cases') and patients without HP (the 'controls'). Regression analyses identified six significant predictors of active HP (Table 2).

Table 2. Significant predictors of hypersensitivity pneumonitis

Variables	Odds ratio (95% CI)
Exposure to a known offending antigen	38.8 (11.6–129.6)
Positive precipitating antibodies	5.3 (2.7–10.4)
Recurrent episodes of symptoms	3.3 (1.5–7.5)
Inspiratory crackles	4.5 (1.8–11.7)
Symptoms 4–8 h after exposure	7.2 (1.8–28.6)
Weight loss	2.0 (1.0–3.9)

The clinical prediction model produced an equation expressing the probability of HP as a function of the statistically significant variables. From this equation, we constructed a table of probability for combinations of predictors (Table 3). In clinical practice, the best diagnostic strategy will depend on the probability of HP determined from Table 3. For instance, in a farmer presenting with recurrent episodes of respiratory symptoms, inspiratory crackles and testing positive for the corresponding precipitating antibodies, the probability of HP would be 81% (Table 3). Another patient presenting with progressive dyspnoea and inspiratory crackles as the unique criteria of HP would have a probability of HP of less than 1%. Further investigation would be mandated only in the former. Typical findings of an alveolar lymphocytosis and/or bilateral ground-glass opacities on HRCT in the former patient would secure the diagnosis of HP, without resorting to surgical lung biopsy. Hypersensitivity pneumonitis would be confidently ruled out in the latter and the investigation oriented towards another diagnosis.

Table 3. Probability (%) of having hypersensitivity pneumonitis*

				Crackles			
				+		–	
Exposure to a known offending antigen	Recurrent episodes of symptoms	Symptoms 4–8 h after exposure	Weight loss	Serum precipitins		Serum precipitins	
				+	–	+	–
+	+	+	+	98	92	93	72
+	+	+	–	97	85	87	56
+	+	–	+	90	62	66	27
+	+	–	–	81	45	49	15
+	–	+	+	95	78	81	44
+	–	+	–	90	64	68	28
+	–	–	+	73	33	37	10
+	–	–	–	57	20	22	5
–	+	+	+	62	23	26	6
–	+	+	–	45	13	15	3
–	+	–	+	18	4	5	1
–	+	–	–	10	2	2	0
–	–	+	+	33	8	10	2
–	–	+	–	20	4	5	1
–	–	–	+	6	1	1	0
–	–	–	–	3	1	1	0

–, absent; +, present; from ref. (4), permission pending.
*All the predictors are dichotomous variables.

Classification of HP

Much confusion still surrounds the classification of HP. Its clinical presentations have classically been defined as acute, subacute and chronic (25). In the acute form, influenza-like symptoms often predominate, consisting of chills, fever, sweating, myalgias, lassitude, headache and nausea that begin 2–9 h after exposure, peak typically during 6 and 24 h, and last from hours to days. Respiratory symptoms such as cough and dyspnoea are common but not universal. The subacute form may appear gradually over several days to weeks, is marked by cough and dyspnoea, and may progress to severe dyspnoea and cyanosis, leading to urgent hospitalization. The chronic form has an insidious onset over a period of months, with increasing cough and exertional dyspnoea. Fatigue and weight loss may be prominent symptoms.

The distinction between the stages of HP is often difficult as they likely represent different manifestations of a single disease that may be related more to the pattern of antigen exposure than to the offending antigen itself. This statement is supported by the finding of considerable overlap in the clinical manifestations of patients with farmer's lung (usually considered as the prototype of acute HP) and those with pigeon breeder's or bird fancier's diseases (the prototypes of subacute and chronic HP respectively (32). Also, chronic HP may still be active and progressive. Others have suggested a classification that takes into account the progression of the disease (acute intermittent, acute progressive, chronic progressive, chronic nonprogressive) that can only be assessed retrospectively (7, 33).

We recently took advantage of the HP study to develop a clinical prediction rule for the diagnosis of HP, to determine whether the current classification of HP (i.e. 'acute', 'subacute' or 'chronic') truly reflects categories of patients with distinct clinical features (34). Data were used to divide a cohort of patients with HP into a limited number of categories ('clusters') with maximally differing clinical patterns, without prejudgement. The results of this cluster analysis were compared with the current classification of HP (acute, subacute or chronic). One hundred and sixty-eight patients were included in the analysis. A two-cluster solution best fitted the data. Patients in cluster 1 (41 patients) had more recurrent systemic symptoms (chills, body aches) and normal chest radiographs than those in cluster 2 (127 patients) who showed significantly more clubbing, hypoxaemia, restrictive patterns on pulmonary function tests and fibrosis on HRCT. Nodular opacities were seen on HRCT as often in cluster 1 as in cluster 2. There was considerable disagreement between the current classification of HP and the results of our analysis. The current classification of

acute, subacute and chronic HP is not supported by our analysis. 'Subacute' HP is particularly difficult to define. Our new classification scheme needs to be prospectively validated however.

Diagnostic methods

Chest radiology

Chest X-ray. Chest radiography is often the initial step in the investigation of a patient presenting with a pulmonary syndrome suggestive of HP. The first objective of chest X-rays is not to rule in HP but rather to rule out other diseases for the patient's illness. In acute HP, one expects to find ground-glass infiltrates, nodular and/or striated patchy opacities (35, 36). The distribution of these infiltrates is usually diffuse but often sparing the bases in the subacute form (37). A variety of different distributions have been described (38, 39). None of these findings is specific of HP. Up to 20% of individuals with acute HP have normal chest X-rays (40).

CT scanning. Our ability to define the usefulness of HRCT in HP is limited by the small number of cases studied. Table 4 summarizes selected reports of HRCT findings according to the phase of disease (41–44). The described patterns are not specific but suggest that HP be considered in the differential diagnosis when present. For instance, ground-glass opacities can be seen in a variety of other diseases including desquamative interstitial pneumonitis, *Pneumocystis carinii* pneumonia, bronchiolitis obliterans with organizing pneumonia, bronchoalveolar carcinoma, alveolar proteinosis and alveolar haemorrhage. Conversely, when ground-glass opacities are associated with poorly defined, centrilobular micronodules and mosaic attenuation or expiratory air trapping, the diagnosis of HP is further supported, but such an association is rare.

Table 4. High-resolution CT findings in hypersensitivity pneumonitis

Stage of disease	References	Sample size	Findings
Acute	Cormier et al. (41)	*n* = 20 (farmer's lung)	Ground-glass opacities Micronodules Mosaic perfusion Emphysema Honeycombing Mediastinal lymphadenopathies
Subacute	Hansell and Moskovic (42)	*n* = 17 (including 9 with pigeon breeder's disease and 4 with farmer's lung)	Generalized increase in attenuation of the lung Nodular pattern Reticular pattern Patchy air space opacification
	Remy-Jardin et al. (43)	*n* = 21 (pigeon breeder's disease)	Micronodular pattern (<5 mm in diameter) Ground-glass attenuation Emphysematous changes Honeycombing
Chronic	Adler et al. (44)	*n* = 16 (antigen = ?)	Fibrosis Ground-glass attenuation Nodules
	Remy-Jardin et al. (43)	*n* = 24 (pigeon breeder's disease)	Honeycombing Ground-glass attenuation Micronodules emphysema

The findings are ranked according to their decreasing order of prevalence in the study population.

Pulmonary function tests

The utility of pulmonary function tests is primarily to describe the physiological abnormalities and the associated impairment. The results of pulmonary function tests may also guide therapy by helping the clinician in selecting those for whom a treatment with corticosteroids may be justified. Pulmonary function tests have no discriminative properties in differentiating HP from other interstitial lung diseases (4).

The typical physiological profile of acute HP is a restrictive pattern with low carbon monoxide diffusion capacity (DLCO) (45). In chronic disease, the pattern can be restrictive, but at least in farmer's lung, the most frequent profile is an obstructive defect resulting from emphysema (46). A currently held belief is that a decreased DLCO is always present in HP (47). Nevertheless, in the HP study, 39 of the 177 patients (22%) in whom DLCO could be measured had normal results (defined as a DLCO ≥ 80% predicted) at the time of diagnosis (HP Study Group, unpublished data).

Specific antibodies

Specific antibodies are not always identifiable in patients with HP, probably signifying that some antigens causative for HP are still unknown. Another unlikely possibility is that HP could occur in the absence of antibodies to the causative antigen. The presence of HP antibodies does not always indicate that the individual has or will develop the disease. In fact, only 1–15% of people exposed to HP antigen develop the disease while in some cases the majority of exposed individuals have a high titre of serum precipitating antibodies but they remain asymptomatic (48). For example, 10% of people exposed to *Saccharopolyspora rectivirgula*, the main agent for Farmer's lung, a well-described form of HP, develop antibodies again the actinomycete while only 0.3% will get the disease (49).

Nevertheless, specific antibodies analysis can be useful as supportive evidence. The results of the HP study demonstrate that positive serum antibodies is a significant predictor of HP (odds ratio: 5.3; 95% CI: 2.7–10.4) (4). Antigens available for testing in most centres included

pigeon and parakeet sera, dove feather antigen, *Aspergillus* sp., *Penicillium*, *S. rectivirgula* and *Thermoactinomyces viridans*. These antigens cover most cases of HP including pigeon breeder's disease, bird fancier's lung, farmer's lung and humidifier lung. The antigen *Trichosporon cutaneum* is also available in Japan for cases of summer-type HP (50). The selection of antigens to be tested often needs to be determined locally according to the prevalent antigens (4, 51). In eastern France, by using a panel of antigens really responsible for farmer's lung and not a classical standardized panel, serological tests showed a high rate for sensitivity and specificity (52).

Several methods for determination of precipitins or total IgG antibodies [immunodiffusion, immunoelectrophoresis, enzyme-linked immunosorbent assays (ELISA)] and different antigen preparations have been described (53, 54). Enzyme-linked immunosorbent assay is usually the preferred method. Unfortunately, even the ELISA technique lacks standardization (55).

Inhalation challenge

Inhalation challenges to suspected environments, usually at the workplace, as well as specific provocation tests in controlled conditions have been described (56). These tests lack standardization both in the inhalation protocols and the criteria defining a positive response. Further studies are needed before recommending inhalation challenges in the diagnosis of HP.

Bronchoalveolar lavage

Bronchoalveolar lavage plays an important role in the investigation of patients suspected of having HP (57). Bronchoalveolar lavage can provide useful, supportive elements in the diagnosis of HP. A normal number of lymphocytes rules out all but residual disease (58). However, the presence of an alveolar lymphocytosis does not, by any means, establish the diagnosis because asymptomatic, exposed individuals can also have increased numbers of lymphocytes in their BAL (59). These individuals do not have subclinical HP as confirmed by a 20-year follow-up (60). Also many other diseases (including sarcoidosis, interstitial pneumonia associated with collagen vascular disease, silicosis, bronchiolitis obliterans with organizing pneumonia, HIV-associated pneumonitis and drug-induced pneumonitis) are characterized by an alveolar lymphocytosis (57). Positive BAL findings [especially if the observed lymphocytosis is marked (61, 62)] in a patient with interstitial lung disease of unknown origin should direct the clinician towards the possible diagnosis of HP (57).

As in the case of serum precipitins and inhalation challenge, the BAL technique lacks standardization. A predominance of $CD8^+$ T lymphocytes and a $CD4^+/CD8^+$ ratio lower than 1 is often observed in HP whereas a high $CD4^+/CD8^+$ ratio is related to sarcoidosis (63) suggesting that this ration could be used to differentiate HP from sarcoidosis. This is now challenged as the CD4/CD8 ratio can be increased in HP to levels as high as those seen in sarcoidosis (64–66). Recent studies suggest that this low $CD4^+/CD8^+$ ratio is associated to the chronic form of HP and to asymptomatic individuals whereas a predominance of $CD4^+$ T cells is related to the acute phase of the disease (67, 68). In addition to the stage of the disease, the $CD4^+/CD8^+$ ratio also depends on the type and dose of inhaled antigen as well as the duration of this antigenic exposure (3, 68, 69).

Lung biopsy

The histopathology of HP has been well described (70–72). In the acute stages, reports on open lung biopsies revealed features of interstitial lymphocytes infiltrates and fibrosis, oedema, noncaseating granulomas, and bronchiolitis obliterans. Macrophages with foamy cytoplasm are also found within the alveolar space. In chronic stages, widespread fibrotic reaction is a prominent feature, often without predominant involvement of upper lobes with contraction. Even though emphysema was found at necropsy in chronic HP, it is only recently that emphysema has been recognized as a long-term complication of HP (46).

Transbronchial biopsy. Haematoxylin–eosin-stained transbronchial biopsy is of limited usefulness for the diagnosis of farmer's lung (FL) (73).

Surgical lung biopsy. The utility of surgical lung biopsy has most often been reported in terms of 'diagnostic yield', i.e. the proportion of specific diagnoses obtained from the procedure. Whether the procedure alters the clinical management represents an important outcome. Several retrospective studies addressing these issues in series of patients with a variety of diffuse parenchymal diseases are available (74–85). In selected reports, the results have been very heterogeneous: the diagnostic yield ranged from 34% to 100%; therapy was altered in 46% to 75% of the cases. This heterogeneity may stem from several factors, including the selection of candidates to open lung biopsy, the timing of the procedure along the course of the disease as well as the expertise of the attending pathologist. The decision to submit a patient to open lung biopsy must be balanced against the associated morbidity. If HP is suspected, it has been our recommendation to reserve surgical lung biopsy for rare cases with puzzling clinical presentation or to verify the clinical diagnosis when the clinical course or response to therapy is unusual (26). This recommendation is not based on evidence but emphasizes the limitations of surgical lung biopsy and the necessity of a thorough clinical investigation that comprises a high index of suspicion and a careful exposure history.

Pathophysiology

The initial data concerning HP pathophysiology come from blood sampling analysis and pulmonary biopsies. High titres of antigen-specific precipitating serum IgG that can fix complement have been observed in patients with HP, supporting the role of an immune complex-mediated reaction. However, cells infiltration, particularly lymphocytes, and formation of granuloma in the lung of HP patients suggest the involvement of a cellular mediated response in the pathophysiology of the disease (67). Over the last decades, the uses of animal models as well as the study of BAL fluid from HP patients has helped understand these processes. There are multiple determinants of HP. These include the type, intensity, and duration of exposure to the offending agent, antigen concentration and solubility, particles size, as well as host susceptibility (69).

Specific antibodies in BAL

Antibodies specific to the offending agent are increased in both serum and BAL of patients with HP (86, 87). Even if the quantity of antibodies in the alveoli correlates with the severity of HP, there is no proof that they are involved in the disease. Studies have shown that inhaled soluble antigens can bind to IgG antibody. This immune complex triggers the complement cascade and the resulting C5 induces macrophages activation, which will release inflammatory mediators (88, 89).

Important actors in HP: inflammatory cells

The role of cells in the pathophysiology of HP has been widely studied. Neutrophils, macrophages, $CD4^+$ and $CD8^+$ lymphocytes, mast cells, natural killer (NK) cells, major histocompatibility class (MHC)-restricted and non-MHC-restricted cells have been found in the lung and BAL of HP patients (3, 90). In most subjects, the normal immune defence mechanisms maintain a homeostasis between antigen inhalation and the host's response.

Neutrophils. Following antigen sensitization, inflammatory cells accumulate in the lung of patients and complex mechanisms involving cells-antigens, cells–cells and cells–inflammatory mediators occur (67). In HP, the nature of the cells present in the lung reflects the stage of the disease. Contact with the offending agent initially leads to a recruitment of neutrophils into the alveoli and the small airways (68). In mice exposed to *S. rectivirgula*, neutrophils amplify the immune response by the release of soluble factors required for the formation of granulomas (91). Elastase, a serine protease, is also produced by neutrophils. In HP this destructive and pro-inflammatory enzyme, which contributes to the break down of elastic fibres could promote the emphysema that has been observed in the chronic form of the disease (46). With activated macrophages, the influx of neutrophils plays a role in the production of oxygen-free radicals, which cause tissue damages and trigger the development of fibrosis also a hallmark of chronic HP (46, 92).

Macrophages. Macrophages also have a preponderant role in the pathophysiology of HP. Following exposure to causal agents, soluble antigens bind to IgG, triggering the complement cascade. The formation of the C5 fraction activates alveolar macrophages which release multiple inflammatory and chemotactic factors such as interleukin (IL)-8, RANTES, CCL18, MCP-1 and MIP-1α contributing to the recruitment of other cells such as neutrophils and macrophages in 4–6 h (93–107). Macrophages recovered from BAL of HP patients show a particular morphology characterized by a foamy cytoplasm (69). Mouse and human macrophages exposed to *S. rectivirgula* release, *in vitro*, tumour necrosis factor (TNF), IL-1 and IL-6 (108). Macrophages from HP patients spontaneously release these cytokines (109). Macrophages from mouse exposed to *S. rectivirgula* express high level of transforming growth factor-beta (TGF-β), an inflammatory cytokine, but a potent inducer of collagen synthesis and fibrosis (110).

Macrophages are normally poor antigen-presenting cells (APC) but this function is greatly increased in HP (103). Following the contact with HP antigen, macrophages incorporate antigenic particles, which will be presented to lymphocytes, triggering their activation and proliferation. Intracellular adhesion molecule-1 (ICAM-1) and B7 co-stimulatory molecules' expressions are increased on macrophages from HP patients supporting a role of macrophages in lymphocytes activation in the pathophysiology of HP (103, 111).

Lymphocytes. Alveolar lymphocytosis is a major characteristic of HP. Lymphocytes are the main cells involved in the pathophysiology of HP and can account for up 60–90% of BAL-recovered cells from patients (61, 112). Lymphocytes usually appear within 24–48 h following the antigen sensitization (68) and their level remains increased for a long time after the cessation of antigen exposure, sometimes even for years. With cessation of exposure, their number however progressively decreases (69).

In HP, the lymphocyte-rich alveolitis is caused by not only a massive recruitment of cells and chemokines, which influence the progression to clinical disease (3), but also by an inhibition of the lymphocyte apoptotic process (113). Lymphocyte-rich BAL may also be observed in asymptomatic exposed subjects. Hence, the presence of this lymphocytosis does necessarily signify the presence of HP and does not predict it eventual development (3).

In HP, lymphocytes express activation markers. Interleukin-2, a cytokine involved in lymphocyte proliferation; IL-2 is also increased in the BAL from HP patients. An over-expression of the IL-2 receptor p75 subunit is also

observed in the patients' $CD8^+$ lymphocytes (3, 63, 104). Finally, lymphocytes are specific to the offending antigen and memory CD45 RO T lymphocytes are found in exposed individuals (114).

CCL18 is a lymphocyte's chemoattractant produced by macrophages. The level of CCL18 is increased in BAL of patients. This is the most abundant chemoattractant produced during the HP subacute phase, which is the state of the disease characterized by the highest lymphocyte count in BAL and a severe inflammation (96).

B lymphocytes involvement is also suggested in HP pathophysiology because of the increased level of antibody, which correlates with the severity of the disease (68).

Other cells. Although lymphocytes and macrophages are the major cells recovered from BAL, other cells also play a role in the pathophysiology of HP. $CD56^+$ NK cells are increased in BAL from HP patients but this level decreases with corticosteroid treatment or antigen contact avoidance (63, 97, 115). Data also show an increased level of mastocytes but their role in HP is still controversial (64, 116, 117). Plasmocytes are also increased in HP with severe alveolitis (118). Dendritic cells could also be involved in HP. These cells are the most potent APCs in the lung (119, 120) and could be implied in the tolerance to antigen in asymptomatic subjects and in the activation of lymphocytes in HP (121).

Soluble factors

Cytokines, chemokines and adhesion molecules all play a critical role in HP by triggering the inflammatory response and recruiting cells. Production and release of IL-1, IL-8, TNF, IL-12, IL-6, MCP-1 and MIP-1α from macrophages are important for the later influx of cells like lymphocytes and the generation of the inflammatory environment (3, 68). These cytokines seems to be responsible for the upregulation of ICAM-1 in macrophages of patients. Upregulation of ICAM-1 acting on lymphocytes through its ligand, lymphocyte function-associated antigen-1, enhances the antigen-presenting capacity of alveolar macrophages in HP patients (111). An increase of B7 co-stimulatory molecules (CD80 and CD86) on macrophages and an upregulation of CD28 on lymphocytes also reinforce the interaction between macrophages and lymphocytes, triggering the lymphocytosis (103). In mice exposed to *S. rectivirgula*, the lung inflammatory response is dependent of interferon-γ and IL-12 and is associated with an upregulation of vascular adhesion molecules (3).

A T helper 1 (Th1) profile of cytokines is secreted in HP. Interleukin-12 secreted by alveolar macrophages promotes the development of Th0–Th1 cells. When the Th1/Th2 balance moves towards a Th1 response, the pathology seems to be more severe. C57Bl/6 mice are more susceptible to HP than other mice strains because they have decreased mRNA stability of Th2 cytokines leading to a Th1 response (122).

Selectins are adhesion molecules involved in the rolling of leucocytes. An increased level of L-selectins is observed in BAL fluid from patients with HP, which is related to the persistence of inflammation (123). The level of E-selectin ligand and L-selectin in BAL correlates with the accumulation of lymphocytes at the inflammation site. In mice exposed to *S. rectivirgula*, a reduction in the inflammatory response and in the lymphocytes count in the lung interstitium is observed if the bond between the L-selectin and its ligand is inhibited (124).

Surfactant

Surfactant is a complex substance containing phospholipids and proteins. Its function is to decrease the surface tension on the alveolar wall. Surfactant can also modulate the immune response. In HP, surfactant is more viscous because its composition is altered. Increased concentrations of phosphatidylanolamine and phosphatidylinositol are observed in patients (125). Also, the surfactant immunosuppressive function seems to be modified in HP. An upregulation of the surfactant protein A, which has proinflammatory properties, is observed in BAL from HP patients (126, 127). However, the level of this protein is not associated with an increased severity of the disease (67). No correlations were seen between levels of surfactant protein A in BAL fluid and numbers of BAL cells, lung function measurements or chest radiographic scores. Moreover, treatment does not cause a decrease of surfactant protein A (126).

Anti-proteases

Levels of anti-protease are increased in BAL from patients with HP (3). The role of these molecules is to protect the lung parenchyma from the proteolytic action of protease such as elafin, secreted from inflammatory cells like neutrophils and macrophages (128, 129). However, the level of anti-protease does not seem to be sufficient to prevent parenchymal destruction (46).

Extracellular matrix

Many components of the extracellular matrix such as type III procollagen, hyaluronic acid, fibronectin, vibronectin and growth factors are involved in lung fibrosis and are increased in HP (46). Some of these markers are correlated with the severity of the disease (130). Thrombin-activable fibrinolysis inhibitor is secreted and produced by lung inflammatory cells and is also increased in BAL fluid from patients. This substance may inhibit plasmin activity leading to an excessive accumulation of extracellular matrix in the lung (131).

Free radicals

During an inflammatory process such as HP, production of the inducible nitric oxide synthase by alveolar macrophages is increased. This enzyme allows inflammatory cells to produce nitric oxide which has direct toxic effects on cells (132). Hence, free radicals and reactive oxygen species could play a major role in the pathophysiology of HP.

Promoting and protective factors

Because only a small percentage of people exposed to HP antigens get the disease, promoting and protective factors may be involved in its pathophysiology. Aetiological agent acting as an adjuvant, concomitant viral infection or genetic predisposition may promote HP in susceptible individuals. Nicotine and some suppressive cells of lymphocytes and macrophages lineage seem to protect from HP.

Aetiological agents. Many HP offending agents are small slowly degradable particles, which explain their retention within the lung. Antigenic substances can interact with complement, antibodies and cells to produce inflammation. This adjuvant effect causes the release of reactive oxygen species, prostaglandins, leukotrienes and proteolytic compounds by leucocytes as well as the production and secretion of IL-1, TNF, IL-12, IL-6, MCP-1 and MIP-1α by macrophages (3).

Viral infection. Many individuals suffering from HP report initial symptoms suggestive of respiratory viral infection at the onset of HP symptoms. Viral antigens are more expressed in lung tissue of HP patients than in normal subjects. A higher concentration of influenza virus protein has been observed in BAL macrophages from patients (133). We have previously demonstrated that mice infected with Sendai virus, a para influenza virus that causes a transient lung inflammation in mice, are more responsive to *S. rectivirgula* antigens. This exacerbated immune response persists for up to 30 weeks after the viral infection (134). A possible mechanism by which a viral infection could enhance HP is by increasing the expression of the CD86 co-stimulatory molecule on APC such as macrophages and dendritic cells. The interaction of the CD86 co-stimulatory molecule on APC with CD28 on T cells is an essential step in the activation of T lymphocytes, cells, which are so abundant in HP.

Genetic predispositions. Genetic markers have generally failed to confirm hereditary risk factors for HP (49, 135–144). Recent studies have shown a polymorphism in the TNF-α^{-308} promoter which is associated with high production of TNF in patients with bird-fancier's lung. Moreover, some MHC class II genes are involved in the positive modulation of the disease (68, 145).

Nicotine. Because HP and specific antibodies are more frequent in nonsmokers, the hypothesis that cigarette smoking may protect from HP has been studied. In fact, smoking habits affect alveolar macrophages phagocytosis and decrease their capacity to produce IL-1 and TNF (33, 146). Nicotine inhibits immunological processes in the lung, decrease total BAL cells like lymphocytes (147). B7 co-stimulatory molecules are also decreased on macrophages exposed to nicotine. In smokers, a viral infection does not increase CD86 molecules expression on macrophages, suggesting a protective role for cigarette smoking in the prevention of HP (103).

Suppressive cells. Dendritic cells are able to instruct T cells response to induce tolerance rather than immunity (148). These tolerogenic dendritic cells are able to drive the differentiation of T-regulatory (Treg) cells, a T-cells subset with suppressive properties. T-regulatory cells have the capacity to actively suppress the proliferation of naïve $CD4^+$ T cells by secreting IL-10 and TGF-β, two effective immunosuppressive cytokines (149). These cytokines can, in turn prevent dendritic cells maturation (150). An increased number of Treg cells could explain the tolerant response observed in mice subjects. The level of IL-10 seems to be lower in patients with diisocyanate-induced HP than in asymptomatic people. Further studies are needed to elucidate this tolerance mechanism.

Animal models

Bronchoalveolar lavage studies have been very helpful in understanding many immunological processes involved in the pathophysiology of HP and is a sensitive tool in the differential diagnosis with others interstitial lung diseases. But, because of limitations in using humans in experimental studies, several animal species have been used to clarify some parts of the pathophysiology.

In the attempt to elucidate the role of T cells in HP, recent studies including adoptive transfer and genetically manipulated mice are performed on animals. Adoptive transfer of antibodies or T cells has been operated in mice. These studies concluded that, in mice, HP is not mediated by serum antibodies but by $CD4^+$ T cells, which interact with recipient cells (3).

Major conclusions have been made following animal models experiments. First, Th1 $CD4^+$ lymphocytes and cytotoxic cells are the most important effector cells in experimental HP. Moreover, animal models confirm that none of the cellular and humoral immunity, soluble factors, mediators, *in vitro* or *in vivo* experiments in humans or animals can fully explain the pathophysiology of HP. A combination of all this processes is necessary to cause the disease (3).

Treatment

The ideal treatment for any form of HP is obviously contact avoidance with the offending antigen. Achieving this objective can sometimes be very simple like getting rid of a parakeet but most often, this solution if far from simple. In poorer parts of the developing countries, the pigeons cohabitate with humans, making the antigenic avoidance impossible to achieve. Farmers are very reluctant to change profession and controlling all antigens from, for example, a dairy barn is very difficult, if not impossible. There are means however of significantly reducing the amount of antigens in the farming industry. These include assuring adequate drying of fodder, using silage instead of hay, avoiding the barns when animals are eating hay, etc. Improving ventilation is also desirable but in cold countries this is limited by the need to preserve indoor heat. With appropriate environmental control most farmers with HP can continue their profession (151). In other work environments like for example wood processing plants, the risk can be decreased by changing different procedures like drying processes and removing moulds on planks before bringing them into the plant *per se* (152). In the homes, it is usually the ventilation system or moulds or indoor birds that are responsible for HP. Appropriate cleaning and preventive measures are usually possible.

The only drugs currently used for HP are oral corticosteroids. These will help control symptoms of acute or subacute bouts of the disease but do not seem to help the long-term outcome (153). When contact can be avoided there is no need for any medication unless the attack is extremely severe compromising ventilation or gas exchange. The dose of corticosteroids to be given when needed is unclear. Most textbooks recommend 50 mg of oral prednisolone daily; others suggest that 20 mg would be sufficient. Low-dose steroids seem as effective as contact avoidance (126). These recommendations are not based on any scientific data but on expert opinions.

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