New aspects of hypersensitivity pneumonitis

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Purpose of review

Hypersensitivity pneumonitis (HP) represents a complex pulmonary disorder of varying intensity and clinical presentation, which is characterized by a diffuse Tc1 immune response of lung parenchyma and airways in patients previously sensitized to one of more than 300 etiologic agents that may favor the HP reaction. This review describes recent data that have clarified some of the events that govern the development of the hypersensitivity reaction following exposure to the causative agents involved in this disease.

Recent findings

A number of recent data clearly demonstrate that several cytokines and chemokines, which are secreted at sites of disease activity, participate in the pulmonary inflammatory responses taking place in the lung of patients with HP.

Summary

The past few years have seen outstanding advances in the understanding of immunologic and molecular events involved in the pathogenesis of HP. It is possible that these data could allow the discovery of therapeutic targets in individuals chronically exposed to HP antigens and evolving towards pulmonary fibrosis.

Keywords

hypersensitivity pneumonitis, immunopathogenesis, chemokines, cytokines

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Introduction

Hypersensitivity pneumonitis represents a group of related inflammatory interstitial lung diseases (ILD) caused by the repeated inhalation of and consequent sensitization to a wide variety of environmental organic dust, fungus, or molds [1,2••]. Thermophilic actinomycetes, omnipresent microorganisms that flourish at the high temperatures reached during the decomposition of vegetables, represent the causative agents that more frequently sensitize exposed subjects. The most common form of HP is farmer's lung first described in Padua in 1713 by Bernardino Ramazzini, who recorded in the *Morbis Artificum Diatriba* his observations associating respiratory disorders with worker exposure to dusts from vegetable fibers and grain. Depending on the host susceptibility, repeated exposure to organic dusts drives an inflammatory reaction within the lower respiratory tract. Lung inflammation is characterized by interstitial inflammation with multiple non-necrotizing granulomas; however, repeated exposure to causative agents may determine the progression toward a severe, irreversible fibrosis. In this review we provide a brief summary on the recent aspects of HP pathogenesis, describing cellular and soluble products that participate in hypersensitivity processes taking place in the lung after antigenic exposure.

Acute phase of hypersensitivity pneumonitis

The exposure to any of a number of organic dusts present in work, home, or hobby environments may result in acute, subacute, and chronic forms of the disease (Table 1). Clinically, acute HP abruptly presents with chills, malaise, fever, dyspnea, and cough. The range of severity can be from a mild influenza-like syndrome to an acute attack of pulmonary edema, needing ventilatory support. The acute episode always follows a previous sensitization, and the intensity of the reaction is related to the amount of inhaled antigen and the duration of exposure. It results in a restrictive and diffusion impairment of lung function and histologically is characterized by an inflammation state involving the alveolar and interstitial structures. Symptoms occur 2 to 9 hours after an exposure, peak typically between 6 and 24 hours, and resolve often spontaneously within 12 hours to several days upon cessation of exposure.

A number of data obtained by the cytologic, immunologic, and molecular evaluation of the pulmonary envi-

Table 1. Clinical presentation of hypersensitivity pneumonitis

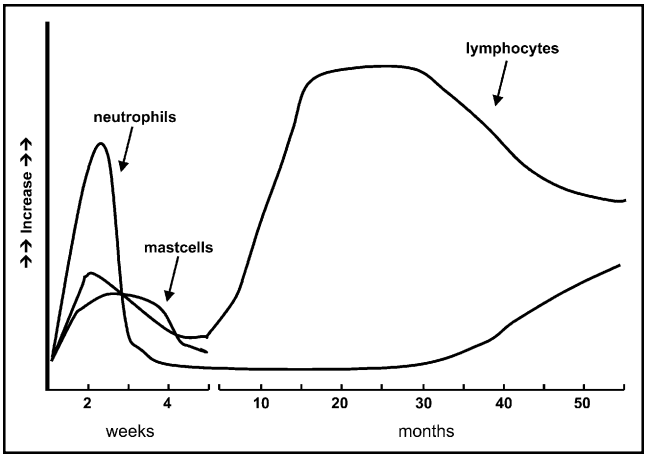
Acute hypersensitivity pneumonitis
- Sudden onset of fever, chills, malaise, cough, dyspnoea, chest tightness, headache, and malaise
- Symptoms are present 4–6 hours following intense exposure to an inciting agent and resolve spontaneously within 12 hours to several days upon stop of exposure.

Subacute hypersensitivity pneumonitis
- Gradual development of productive cough, dyspnoea, fatigue, anorexia, and weight loss.
- Findings are present in patients who experience repeated acute attacks.

Chronic hypersensitivity pneumonitis
- Insidious onset of cough, progressive dyspnoea, fatigue, and weight loss
- Patients may lack a history of acute episodes.
- Removing exposure results in only partial improvement.

ronment by bronchoalveolar lavage (BAL) and induced sputum [3] have shown that immune cells involved in the acute process include neutrophils, mast cells, lymphocytes, and monocyte-macrophages (Fig. 1). Soon after inhalation of antigen, neutrophilic inflammation is prominent in the lungs. In fact, it is known that acute neutrophilia may be demonstrated in the bronchoalveolar lavage after inhalation of antigen, followed by lymphocytosis. *In vitro* data suggest that respiratory epithelial cells may play a role in this phenomenon *via* the production of chemokines, such CXCL8/IL-8 [4]. Epithelial cells exposed to thermophilic bacteria release chemokines in a dose- and time-dependent manner that is blocked by both corticosteroids and IL-10. Causative antigens also trigger expression of the DNA-binding activity of NF-kappa B, a transcription factor that mediates activation of the IL-8 gene. Furthermore, results obtained in C57BL/6 mice either lacking the TNF-α class I receptor (TNF-alphaRI(–/–)) or treated with neutralizing anti-TNF-α monoclonal antibodies demonstrated

Figure 1. Trends of bronchoalveolar cell populations during different clinical phases of hypersensitivity pneumonitis



A number of data obtained by the cytologic, immunologic, and molecular evaluation of the pulmonary environment by bronchoalveolar lavage (BAL) and induced sputum [3] have shown that immune cells involved in the acute process include neutrophils, mast cells, lymphocytes, and monocyte-macrophages.

an essential contribution of TNF-α in mediating neutrophil influx and hemorrhage [5]. There are also data in humans indicating that a genetic predisposition to TNF-α production and a consequent increased production of TNF-α after contact with hay are implicated in the pathogenesis of alveolitis in farmer's lung [6].

The lymphocytosis develops after a variable time from the acute exposure and, as better specified in the following paragraphs, is largely the result of the recruitment and/or the local proliferation of CD8+ T cells in response to well-defined antigens as in the case of summer-type hypersensitivity pneumonitis (SHP) [7•].

Subacute phase of hypersensitivity pneumonitis

In the subacute form of HP, the disease is more insidious and is usually characterized by a bronchitis and the absence of acute features. In subacute disease sputum production, cough, anorexia, and weight loss are commonly observed. Often an important anamnestic finding is the dramatic improvement in symptoms when the patient remains away from the exposure environment. Lung involvement in subacute HP is characterized by a lymphocytic alveolitis with an increase in both percentage and absolute number of CD8+ T cells in the BAL. These cells are immune suppressor/cytotoxic cells, which display suppressor activity, are able to kill NK sensitive targets [8], and show an oligoclonal pattern of growth [9]. The evidence that identical T cell clones are present in the lung and the blood of the same patient suggests that the immune reaction occurring at lung level gives rise to a systemic reaction [10••]. Lung CD8+ cells also bear at high density molecules of a superfamily whose members interact with a parallel family of ligands showing homology to tumor necrosis factor (TNF), such as TNF-receptor type 2 (CD120b), Fas/CD95, and CD70 [11,12•]. Furthermore, they express IFN-γ and functional capabilities, which are typical of Tc1 lymphocytes [13].

Macrophages represent the second largest cell population obtained by BAL from the lungs of patients with HP. Their absolute number is often increased. These cells are activated, as demonstrated by the significant expression of activation markers including CD54, selectins, and co-stimulatory molecules [14]. However, there are data indicating that L-selectin is upregulated in macrophages and neutrophils while E-selectin is overexpressed by endothelial cells [15••]. Thus, it is likely that adhesion molecules that contribute to leukocyte recruitment into the tissue after an injury are upregulated during the development of symptomatic HP, playing a role in the homing of both macrophages and neutrophils. Data obtained in the animal model confirm the role of macrophages in inducing HP reaction [16••]. A number of macrophage factors are released in sensitized mice

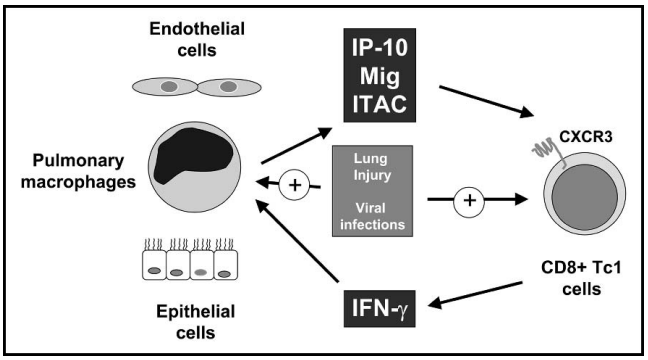
including IL-1 and IL-12, which enhance lymphocyte expansion and promote Th1 differentiation, resulting in hypersensitivity pneumonitis.

An increase in the macrophage expression of B7 family members (CD80 and CD86) can be found in the lung of HP patients [17]. Considering the role of these molecules in the T-cell/accessory cell interactions and in the control of lymphocyte proliferation in the lung [18], it has been suggested that CD80 and CD86 are important regulatory components in the development of lymphocytic alveolitis. The importance of these molecules is highlighted by results showing that blockade of CD28/B7 interactions by CTLA4-Ig inhibits hypersensitivity inflammation in mice lungs exposed to HP Ag [19]. There are also data on the putative mechanisms that favor the expression of B7 molecules: viral infections can enhance hypersensitivity reaction by increasing B7 expression. Thus, it is possible that some viruses may induce the development of hypersensitivity pneumonitis in predisposed individuals [20••], explaining the recurrence of HP episodes during viral superinfections.

Recent data involve macrophages as a cell source of chemokines that drive T-cell recruitment. Pardo *et al.* [21] have recently examined the expression of dendritic cell (DC)-derived CC chemokine 1 (CK1)/CCL18 in lungs of patients with HP. CCL18 expression is significantly increased in lungs affected by HP, and its levels are higher in the subacute rather than in the chronic phase of the disease. Macrophages, dendritic cells, and alveolar epithelial cells are the main sources of CCL18. Interestingly, a direct correlation between the levels of tissue CCL18 and the number of lymphocytes may be demonstrated in the bronchoalveolar lavage fluids. Chemokines, in particular interactions of CXCR3 with their ligands (CXCL9, CXCL10, and CXCL11), are also likely to be involved in the recruitment of lymphocytes and granuloma formation [22•]. These findings suggest a role for chemokines in the pathogenesis of HP. Since viral infection may induce the production of Th1/Tc1 chemokines *via* the production of IFN-γ, it is possible that viral superinfections, by upmodulating chemokines that favor the development of hypersensitivity reactions, support the persistence of the local inflammatory processes (Fig. 2).

As recently reviewed, a number of antagonists for chemokine receptors are being developed by different pharmaceutical companies [23]. It is hoped that antagonists of chemokine receptors may be quickly approved for human trials. In fact, the therapeutic use of molecules selective for chemokine receptors appears to have great potential for all the inflammatory disorders that are characterized by a massive accumulation of T cells within affected organs, including HP.

Figure 2. Viruses able to upregulate production of chemokines



Viruses are able to upregulate the production of chemokines that are involved in the recruitment of CD8+ Tc1 cells at sites of inflammation. Induction of chemokines including IP-10, Mig, and ITAC may represent a key mechanism that favors the development of CD8+ T cell alveolitis in hypersensitivity pneumonitis. Newly recruited CD8+ Tc1 cells are able to release IFNγ and this might in turn further upregulate chemokine production by pulmonary macrophages as well as by non-immunocompetent cells including epithelial and endothelial cells.

Chronic phase of hypersensitivity pneumonitis

The chronic form of the disease is characterized by progressive dyspnea without acute episodes or systemic features and is caused by the prolonged exposure to small quantities of the responsible antigen. Patients may lack a history of acute episodes. Lung histology in these patients shows interstitial pneumonitis, granulomas, and fibrosis, pathologic findings that depend on the stage of the disease at tissue sampling [24]. Removing exposure results in only partial improvement since HP may evolve into a diffuse, fatal fibrotic disorder in patients with subacute/chronic disease. For instance, it has been recently shown that some improvement could be seen in most individuals with subacute and chronic form of pigeon breeder's disease (an avian-induced hypersensitivity pneumonitis), though recovery may still not be complete [25•]. In particular, those with the chronic form are at high risk for morbidity. As in other ILD characterized by a fibrotic evolution, a chronic neutrophilia is usually observed in chronic HP [26]. Specifically, in HP tissue samples neutrophils are located inside vessels, and in the interstitial and alveolar spaces. Tissue neutrophils show intense immunoreactive collagenase-2 and gelatinase B staining [26], suggesting that in subjects who are chronically exposed to HP antigens there is a persistent traffic of activated neutrophils that may play a role in the lung damage and, thus, in the fibrotic evolution.

Other aspects of hypersensitivity pneumonitis reaction

Immune abnormalities can be demonstrated not only in symptomatic subjects with HP but also in asymptomatic individuals exposed to HP antigens [27]. In fact, a T-cell alveolitis can be observed in exposed subjects with normal chest x-rays. These people are commonly defined as

having asymptomatic HP, and it is important to note that the CD8 T-cell count returns to normal levels in individuals who are not further exposed to the specific antigens that are responsible for the development of HP alveolitis. The reason why some exposed subjects do not develop a symptomatic and persistent disease while others show a progressive and invaliding disorder is unknown. It has been suggested that a Th1/Th2 dysregulation may occur only in exposed patients who develop a symptomatic HP. Th1, but not Th2, cell lines can adoptively transfer experimental hypersensitivity pneumonitis (EHP) [16]. However, data obtained in animal models of hypersensitivity pneumonitis have clearly shown that Th1-biased C57BL/6 mice are susceptible while Th2-biased DBA/2 mice are resistant to HP; decreased mRNA stability of Th2 cytokines seems to be important in explaining the increased susceptibility of this mouse strain [28••]. A tempting idea is that exposed individuals who develop a frank HP have a genetically determined Th1/Th2 dysregulation.

The application of BAL also provided interesting information on the natural history of the disease in children. Hypersensitivity pneumonitis may be an underrecognized form of immune-mediated ILD in children exposed to the inhalation of organic antigens [29]. BAL evaluation may be of value in diagnosing pediatric HP. Ratjen *et al.* [30•] have shown that the percentage of lymphocytes is significantly increased in all pediatric patients with HP, although no significant differences can be observed in the lung CD4/CD8 ratio between children without lung disease and those with HP. Furthermore, the authors suggest that assessing natural killer cells and human leukocyte antigen-DR expression is a helpful adjunct in the diagnosis of pediatric patients with this disorder.

Perspectives

A biologically based and potentially powerful way to characterize human diseases has been found in the new science of genomics, which might help scientists to discover gene function, determine biologic pathways, and better understand the pathogenesis of several interstitial lung diseases, including HP [31]. It is today possible to evaluate which genes are expressed in a particular cell type, at a specific time, under circumstantial conditions, and during different phases of a pathologic condition. Initial data on HP patients have been obtained with this tool [32•]. The expectation is that the evaluation and in depth analysis of thousands of genes in large numbers of lung biopsies and bronchoalveolar lavage specimens obtained from patients with HP might provide a detailed molecular description of the natural history of inflammatory events taking place in the pulmonary microenvironment during this intriguing disease. However, the use of microarrays could become an essential tool for evaluating genetic susceptibility to HP. It is also possible that these innovative tools could lead to the discovery of new therapeutic targets and the identification of predictive markers, simplifying the prediction of response to treatment and clinical outcomes in exposed subjects with subacute/chronic disease.

Conclusion

Although an impressive amount of detailed information on the immunopathogenesis of HP are available there is clearly much more to be learned. More research is required to define the actions of cytokines on granuloma formation and pulmonary tissue injury on a molecular basis. It is easy to predict that by taking advantage of new powerful ways to characterize human diseases, as genomics and proteomics, the near future will be characterized by further progress in the identification of molecules that mediate lung damage and thus clinical history of HP.

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