Hypersensitivity Pneumonitis; State of the Art*

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We should like to limit the term hypersensitivity pneumonitis to include only those alveolar filling and interstitial diseases produced after intense or prolonged exposure to finely dispersed organic dusts (animal protein, actinomycete or related antigens) of appropriate particle size.

Although these diseases are ill defined, they are characterized by several common features:

1. They seem to involve the peripheral airways exclusively and features such as hilar lymphadenopathy, pleural effusion, or systemic involvement, as has been noted in sarcoidosis, are not present;

2. Histologically, they consist of mononuclear cell, interstitial and alveolar infiltrates;

3. Granuloma formation has often been a prominent histologic feature;

4. Large numbers of cells with the characteristics of activated alveolar macrophages are found within the lung lesions;

5. They are associated with the development of high titer precipitating antibodies against the offending organic dust antigen;

6. They are not associated with changes in serum complement activity (although the etiologic agents involved can activate complement via the alternative pathway);

7. They are associated with elevated serum levels of IgG, A and M, but IgE levels are normal and there is no eosinophilia;

8. In vitro correlates of cell-mediated or delayed hypersensitivity indicative of lymphokine production are usually positive in symptomatic patients.

Etiologic Agents

A large number of occupational, avocational and iatrogenic sources of antigen are being recognized continuously. These include antigens derived from microorganisms (bacteria, fungi, Actinomycetes and amoebae), animal protein, and plant protein. Certain small molecular weight chemicals and drugs may also produce the disease. It has been well established that the antigenic load of these substances is heavy. For example, it has been estimated that a farmer, working in an environment where aliquots of moldy hay have been disturbed, might inhale and retain approximately 750,000 Actinomycetes spores per minute in his lungs. Antigens of Actinomycete and avian origin have been the most extensively studied. The antigenic components of *Micropolyspora faeni*, the most important source of Actinomycete antigen in moldy hay, have been studied over the past several years by Roberts, Edwards, and others.1,2 Edwards originally identified eight major antigens by immunoelectrophoresis and labeled them according to electrophoretic mobility. Purification and immunochemical analysis revealed that among these antigens were several heat-stable glycopeptides with molecular weights of approximately 85,000 daltons, a heat-labile protein with molecular weight of 44,000 daltons demonstrating chymotrypsin-like activity, another heat labile protein with molecular weight of 77,000 daltons and demonstrable enzymatic activity, plus a rapidly anodally migrating heat labile protein with high sugar content and molecular weight of 101,000 daltons. More recently, Roberts, using the discontinuous gel gradient polyacrylamide technique, identified over 30 different protein bands with different electrophoretic patterns for each of several actinomycete species detected. Again, several of these proteins demonstrated enzymatic activity.

Avian antigens have also come under considerable study in the past few years in hypersensitivity pneumonitis associated with exposure to pigeons and parakeets. A recent extensive study by Fredericks3 using a soluble, undialyzed pigeon dropping extract yielded four major antigens on immunoelectrophoresis. One of these antigens, a glycoprotein with a molecular weight of 200,000 daltons, was shown likely to be a pigeon IgA molecule derivative that survived and resisted enzymatic degradation in avian excreta. Another of these antigens was susceptible to treatment with mercaptoethanolurea and appeared to be a mucopolysaccharide protein conjugate. Yet another glycoprotein with molecular weight of 43,000 daltons was thought to be a Fab or Fc breakdown product of pigeon IgA. Thus, in the case of Actinomycete antigens, an intensive study is currently in progress to elucidate the molecular and biologic properties of these potent avian antigens.

As can be seen from these studies, the biologic activity and characterization of Actinomycete and avian antigens is becoming increasingly complex and a multiplicity of different antigens, many of which possess enzymatic activity, are being defined. Such studies will continue to be of importance since the development of accurate diagnostic tests for these diseases will depend upon well characterized, purified and standardized antigens.

Clinical Features

There have been some recent new insights into the clinical presentation of these diseases and it is becoming increasingly apparent that the intensity and frequency of exposure to etiologic antigens determines the clinical categories of acute and chronic forms of the disease. The importance of obtaining a careful occupational history remains paramount in diagnosis, as is the realization that...
the classic symptoms of cough, fever, chills, malaise and dyspnea are often mistaken for a bacterial or viral pneumonia. More recent information is available, however, indicating that these diseases, when left unrecognized, can lead to irreversible lung damage after repeated episodes. The discovery of humidifier lung by Fink and co-workers has also made us aware of the fact that, in contrast to job related occupational symptoms, exposure to Actinomycete contamination of humidifiers may occur in a non-occupational setting.

Little is known about the natural history and prognosis of the disease in the face of chronic exposure, but in a study of farmer's lung patients, a five-year morbidity rate of 30 percent was reported with respiratory disability due mainly to pulmonary fibrosis. Thus, it can be recognized easily that this disease may have tremendous socioeconomic impact with severe personal hardship and loss of time from work.

Diagnosis

The first diagnostic step is to establish an association between inhalation exposure to an organic dust or animal protein and the development of pneumonitis. Although immunologic laboratory procedures may be additionally useful in establishing a specific diagnosis, many of these are unfortunately still limited to the academic environment because of the technology required. Even when such tests are performed with proper controls, they do not always differentiate between individuals with overt disease and those who have been exposed but remain well. Most subjects with hypersensitivity pneumonitis demonstrate serum antibodies which specifically precipitate with the offending organic dust, as has been well described for many years. These antibodies are easily detectable by conventional Ouchterlony double diffusion analysis. In general, only poorly standardized crude extracts of Actinomycete species, certain fungi, crude organic dusts and avian proteins are commercially available for patient screening and the need for better characterized and purified antigens remains. This is emphasized by a recent NIAID-sponsored symposium which was devoted entirely to the development of better diagnostic reagents for these diseases.

There are problems with false negative precipitin tests that occasionally occur, possibly because any given batch of diagnostic antigen may be insufficiently pure or potent. False positive precipitin tests also continue to occur and there continue to be many attempts to employ more sensitive screening tests for disease diagnosis. Unfortunately, considering the current availability of impure antigens, increased sensitivity invariably leads to further decrease in specificity. Nonetheless, since commercial antigens are poorly standardized and the list of offending antigens is constantly expanding, it is reasonable for screening purposes to check any suspected organic dust for antigenic activity by simple Ouchterlony double gel diffusion using appropriate controls. If positive, the crude dust extract can be presumed to contain an offending antigen, and further precipitin tests can be performed with more purified antigenic components.

In addition to precipitin studies, there remains a need for good skin test antigens in these diseases. Although extensively dialyzed non-irritating Actinomycete extracts have been successfully used for skin testing in experimental animals, it is apparent that after many years, crude Actinomycete preparations currently available are still nonspecifically irritating in man. Some animal protein antigens, on the other hand, such as pigeon serum can be used for skin testing in man. These antigens produce positive late (four to eight hours) presumed Arthus type reactions and immediate (20 minutes) wheal and flare reactions.

Several studies over the past few years have also continued to focus on the production of lymphokines such as MIF and blastogenic factor by peripheral blood leukocytes and bronchoalveolar lymphoid cells in patients with symptomatic disease. Such lymphokine production appears to correlate with disease activity. These in vitro assays can be very useful diagnostically when considered along with other clinical laboratory data. Unfortunately, the use of these techniques is generally limited to academic centers.

Pathology

Although there have been several recent exciting immunofluorescent and histopathologic findings in the idiopathic fibrotic pulmonary diseases, immunofluorescent studies in hypersensitivity pneumonitis continue to be generally unrewarding. Most varieties of the disease are characterized by similar histologic changes which largely depend upon the intensity of antigenic exposure and the stage of the disease at the time of biopsy. The basic tissue reaction consists of alveolar and interstitial inflammation often with marked involvement and narrowing of bronchial walls characteristic of bronchiolitis obliterans. The infiltrates classically consist of lymphocytes and plasma cells along with large numbers of alveolar macrophages which are activated. In the subacute forms of the disease, non-caseating granulomata, which resemble those found in sarcoidosis, are often prominent. With further passage of time, granulomatous lesions may persist or disappear as interstitial fibrosis develops. In contrast, the majority of acute episodes following single brief exposures usually resolve without pulmonary residua. The walls of bronchioles have been shown to stain with fluoresceinated globulins isolated from patients with the disease, indicating the presence of Actinomycete antigen in this location. IgG, IgA and IgM have been found on plasma cells and lymphocytes scattered in and about the granulomatous lesions, and complement products have been demonstrated on the surface of histiocytes. Pulmonary vasculitis does not appear to be a consistent feature but there remain isolated reports of biopsy-proven vasculitis during acute episodes.

Pathogenesis

The most intriguing recent studies of the disease have been in the area of pathogenesis. The antigens involved
have been shown to exert several important biologic effects including the following: nonspecific activation of serum proteins (complement alternate pathway) and cells (alveolar macrophages); immune response enhancement (a nonspecific adjuvant effect); a specific humoral and cellular immune response (serum antibody and lymphokine production); and tissue destruction (macrophage lysosomal enzyme release).

Determinants of host susceptibility are poorly understood but they clearly exist since only a minority of regularly exposed subjects contract the disease. Although data are conflicting, there is emerging evidence for genetically-determined susceptibility. Certain HLA antigens, such as HLA B8, have been found with increased frequency in farmer's lung and pigeon breeders' disease. HLA-BW40 has also been found with increased frequency in pigeon breeders' disease. Since the genes coding for these antigens are closely linked to immune response (IR) genes in the major histocompatibility complex, the increased frequency of this B-locus allele may suggest the presence of an IR gene governing abnormal reactivity to these etiologic antigens. Apart from their antigenic property, it is becoming increasingly clear that certain offending agents have nonspecific irritating effects. Recent animal models seem to indicate that nonspecific lung inflammation augments pulmonary sensitization as a result of the known adjuvant properties of inhaled Actinomycete antigen. Other recent studies have shown that induction of nonspecific inflammation of the lung with BCG, followed by inhalation challenge results in granulomatous lesions resembling those of hypersensitivity pneumonitis and demonstrable cell-mediated hypersensitivity to the inhaled antigen, whereas longterm immunization with certain of these crude antigens results only in antibody formation without inflammatory lesions. This finding would tend to indicate that offending inhaled antigens alone are not sufficient to induce pulmonary lesions unless local cell-mediated immunity is initially present. Of greater interest is the specific immune response to etiologic antigens in hypersensitivity pneumonitis where a host of recent animal models and human studies have greatly contributed to our understanding of the disease process. It is now well established that atopy is not a feature of these diseases and IgE levels and eosinophil counts are typically normal. Type II cytotoxic mechanisms may play a role in disease pathogenesis as demonstrated by the findings of Wenzel and co-workers who demonstrated Actinomycete antigen, immunoglobulin, and complement in bronchial walls and mononuclear cells of lung biopsy specimens from farmer's lung patients. They speculated a direct complement and immunoglobulin mediated cytolytic effect, particularly with regard to alveolar macrophages that have adsorbed antigen in their surfaces.

Evidence for type III immune complex-induced injury is stronger and has traditionally represented the conventional wisdom in discussing pathogenesis of this disease. The presence of specific precipitating antibodies which correlate with the intensity of exposure, the development of late Arthus type skin reactions with some antigens which are at times histologically characterized by necrotizing vasculitis, and the production of symptoms within a four to eight hour latent period following natural exposure or bronchoprovocation, argue for participation of this type mechanism. In addition, immunofluorescence studies of lung biopsy material have been reported to demonstrate antigen, immunoglobulin, and complement, all the necessary constituents of an immune complex reaction. These facts not withstanding, there is considerable evidence that type III immune complex induced tissue injury is not the major pathogenetic mechanism operative in this disease. For example, precipitins are present in a large percentage of exposed asymptomatic subjects; the histopathology of the lung lesions in most cases of hypersensitivity pneumonitis is substantially different from the vasculitic lesions in immune complex disease; and serum complement levels which usually drop in acute immune complex disease either remain within the normal range or increase following natural exposure or bronchoprovocation challenge of these patients. Indeed, decreases in serum complement seem to occur in asymptomatic rather than symptomatic subjects following antigen challenge. More recent evidence does, however, indicate that organic dust antigen directly activates the alternative complement pathway in vitro and it is known that certain of these antigenic components can consume hemolytic complement through a mechanism that apparently involves nonspecific activation of both the classic and alternative pathways. Thus, it is possible that some early nonspecific inflammatory components are based on alternative pathway activation.

Since the pulmonary histopathology of hypersensitivity pneumonitis greatly resembles tuberculin-like cell mediated type IV hypersensitivity, many recent animal model studies have focused on defining a possible contribution of this type hypersensitivity in the pathogenesis of the disease. Studies in man have shown that lymphokine production (migration inhibition and blastogenic factors) occurs after peripheral blood lymphocyte exposure to avian and Actinomycete antigens. In pigeon breeders' disease, lymphokine production appears to characterize most symptomatic individuals, while asymptomatic exposed subjects demonstrate no reactivity. Lymphokine production has also been demonstrated in recent animal models of this disease involving several aspects. Of particular significance is the finding that transfer of specifically sensitized lymph node cells, but not serum, into previously unexposed rabbits, followed by aerosol challenge, produces disease.

Of importance are the recent findings that local organ restricted immune mechanisms may be operative in the lung with or without systemic manifestations of sensitization. A recent report indicates that bronchoalveolar cells from patients with pigeon breeders disease produced lymphokines in response to appropriate antigen, while peripheral blood lymphocytes taken concurrently from the same subjects did not. A higher proportion of lymphocytes, higher IgG and IgM levels, and higher
T/B cell ratios have also been found in bronchial washings of patients with hypersensitivity pneumonitis when compared with other control individuals. All of these data indicate that the immunologic reactivity of the lung may not necessarily parallel that noted in the skin or peripheral circulation. Thus, although the classic Gell and Coombs classification of hypersensitivity has been conceptually useful, its admitted artificial separation of related immune functions may not be applicable to the lung. This may in part explain why it has been so difficult to fully characterize the pathogenesis of hypersensitivity pneumonitis in terms of discrete mechanisms of allergic tissue injury.

Studies of cells and fluid obtained in both humans and animals by bronchial lavage have also recently received attention in these diseases. A series of recent studies has shown that repeated respiratory tract exposure to Actinomycete antigen leads to the development of pulmonary lesions comparable to those of human hypersensitivity pneumonitis, early lesions being composed predominantly of interstitial, peribronchial, and intra-alveolar mononuclear cells and later lesions characterized by granulomata and giant cells. In these studies, the large number of bronchoalveolar cells obtained by lavage demonstrated few phagolyosomes suggesting that they were immature phagocytes recruited in response to lung injury. These lesions also contained large numbers of alveolar macrophages that were activated morphologically, metabolically, and with regard to phagocytic and bactericidal activity. Of importance in this regard is the fact that the intense granulomatous pulmonary infiltrates, as well as the presence of activated alveolar macrophages could be specifically recalled by challenge with Actinomycete antigen indicating that macrophage activation is at least in part an immunologically specific event.

In attempting to correlate all of these data and events, one might propose the following hypothesis. Most organic dusts are efficient activators of the alternative complement pathway and it may be that some of the initial inflammatory effects of inhaling these agents are nonspecific in nature and mediated via this mechanism, as well as a non-specific adjuvant effect. Complement split products such as C3b could induce lysosomal enzyme release from alveolar macrophages and these enzymes, which have been shown to cleave C3 and generate more chemotactic complement products as well as C3b, would thus lead to further recruitment and activation of macrophages forming an amplification loop of inflammation. It is conceivable that pulmonary granulomas containing large numbers of activated macrophages with ingested particulate antigens could evolve through this type of mechanism. Thus, this hypothesis for development of hypersensitivity pneumonitis would require initial nonspecific inflammation such as activation of the complement system by inhaled antigen with the subsequent generation of chemotactic factors and ultimate granuloma formation or fibrosis. Immunologically-specific events would also be operative in this scheme. Sensitized B lymphocytes and plasma cells would produce antibody, and continued inhalation of antigen would result in formation of immune complexes which in turn would be ingested by macrophages and lead to their activation. Additionally, available evidence would indicate that sensitized T cells also participate in the pathogenetic process by secreting lymphokines such as MIF or blastogenic factor which in turn would produce further macrophage activation. Thus, the central event in production of the lesions would be the activation of the alveolar macrophage. It is even possible that persistent inflammation would result in fibrosis through a macrophage-mediated influence on fibroblasts by soluble mediators, as has been proposed by Schorlemmer and co-workers. According to this hypothesis, the activated alveolar macrophage would not only remain the pathogenetic focal point, but would serve as a functional link between an inhaled offending antigen and the consequent production of pulmonary lesions. This hypothesis also allows for the participation of immunologically specific and nonspecific events, as well as the participation of both B cell products (antibody or immune complexes) and T cell products (lymphokines in cell mediated immunity) in disease pathogenesis.

REFERENCES

Experimental Hypersensitivity Lung Disease

Chronic Pulmonary Inflammation and Cell-Mediated Hypersensitivity by Exposure to Aerosolized Antigens in Rabbits*

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In the group of diseases termed hypersensitivity pneumonitis (HP), lung lesions generally display chronic interstitial and alveolar granulomatous inflammation.1 Because of this pathology, it has been suggested that cell-mediated hypersensitivity (CMH) contributes to the pathogenesis of the group of diseases.1 In addition, other studies using blood and lung leukocytes have added impetus to this thesis.2,3 Chronic granulomatous pulmonary inflammation is best produced experimentally by the injection of particulate antigens such as BCG, Micropolyspora faeni, and antigens attached to large carriers such as Sepharose 4B. Attempts to produce chronic lung lesions in animals using soluble aerosolized antigens have been difficult.4 In the present study, we present evidence that chronic pulmonary inflammation can be produced in immunized animals after chronic exposure to aerosolized, soluble antigens. In addition, the inflammation was associated with CMH in bronchoalveolar cells (BAC), was immunospecific, and was not transferrable to normal rabbits with large quantities of immune serum.

MATERIALS AND METHODS

New Zealand white rabbits of either sex were used in these studies. They were immunized subcutaneously in the inguinal area with 4 mg of antigen in complete Freund's adjuvant (CFA). Two weeks later, the animals were given booster injections as in the initial immunization procedure. Some control animals were immunized and given booster injections with only CFA.

Two weeks after the booster injection, the animals were insufflated five days per week for three weeks with approximately 50 ml of 1 mg of antigen/ml in an ultrasonic nebulizer DeVilbis 65; this instrument delivers particles ranging from 2-10 μm in diameter.

Some animals were evaluated after immunization, but prior to insufflation for systemic CMH, CMH in the lung using BAC and migration inhibitory testing (MIT), for antibody activity in serum by passive hemagglutination (PHA) and tanned cell hemolysis (TCH), the latter a test for complement-fixing antibodies. Other animals were evaluated for these same parameters two days after their last aerosol exposure to aerosolized antigen.

Lungs from animals were evaluated histologically after immunization and booster injections, and two days after their last exposure to aerosolized antigens.

RESULTS AND DISCUSSION

Animals immunized and given booster injections (but not insufflated) developed high antibody activity in the blood as assessed by either PHA or TCH. These animals also developed activated lymphocytes in the spleen that were capable of incorporating increased quantities of 3HTdR in the presence of antigen. However, immunization did not result in the induction of CMH in BAC (only one animal of nine tested was positive). Thus,