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Detection of antigen-specific antibodies on lung tissue in a patient with hypersensitivity pneumonitis

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Summary. A patient with hypersensitivity pneumonitis showed positive Ouchterlony's immunodiffusion tests against pigeon faecal extract, Cephalosporium and Pullularia antigens. Deposits of immunoglobulins – IgG and IgM antibodies – were detected in a subendothelial position in arterial and venous vessels and on alveolar macrophages in the lung tissue. The IgG desposits in blood vessels belonged to IgG_1, IgG_2 and IgG_3 subclasses and the absorbed IgG on alveolar macrophages to all IgG subclasses. The detection of allergen specific antibodies in lung tissue was made by indirect immunofluorescent staining with FITC conjugated antigen extracts from pigeon faeces and demonstrated the aetiology of this hypersensitivity pneumonitis.

Key words: Hypersensitivity pneumonitis – Indirect immunofluorescent staining – Antigen-specific antibodies

Introduction

Hypersensitivity pneumonitis (HP) – also called extrinsic allergic alveolitis – is a feature of the complex immunological reaction of the lung to a wide range of inhaled organic dusts and other antigens (Braun et al. 1986; Salvaggio 1987; Salvaggio and Karr 1979). High levels of serum-precipitating antibodies against the appropriate offending organic antigens are associated with hypersensitivity pneumonitis but are also detectable in some exposed but asymptomatic individuals (Müller et al. 1987; Phanuphak et al. 1975). The histological features of HP vary, from the presence of lymphocytes, plasma cells, monocytes, neutrophils and foam cells in the lung interstitium and alveolar walls to the presence of epitheloid non-caseating granulomas with giant cells (Johnson et al. 1979; Kawanami et al. 1983). The immunological characteristics are decreased helper/suppressor cell ratio and increase of class 2 histocompatibility antigen (HLA-DR) bearing T-lymphocytes (Bauer et al. 1985; Keller et al. 1984; Mornex et al. 1984; Popp et al. 1987). Bronchoalveolar lavage fluid contains increased levels of IgG, IgM and IgA, and absorbed immunoglobulins can be detected on alveolar macrophages; there are also deposits of immunoglobulins in a subendothelial position in blood vessels in the early phase of these diseases (Braun et al. 1986; Ghose et al. 1974; Naegel et al. 1984; Roberts and Moore 1977). Even when there is extensive pathological change, no single morphological feature is diagnostic. The diagnosis of HP is therefore often based on a suggestive history and the exclusion of other causes (Johnson et al. 1979). This study demonstrates pathophysiological changes and the detection of antigen specific antibodies on lung tissue in a patient with different serum-precipitating antibodies. This permits confirmation of the aetiology of the disorder.

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Abbreviations: HP, hypersensitivity pneumonitis; FITC, fluorescein-isothiocyanate; PBS, phosphate buffered saline

Materials and methods

A 23 year old woman who lived under bad social conditions in a damp room near to a pigeon-house, had a history of multiple bouts of dyspnoea, wheezing and malaise. Chest roentgenogram showed a diffuse reticulonodular pattern of an interstitial and alveolar filling process. Pulmonary function test confirmed a long-standing restrictive lung injury with a decreased diffusion capacity for carbon monoxide. Serum-precipitating antibodies to pigeon faecal extract, Cephalosporium and Pullularia antigens (Hollister-Stier) were detected by Ouchterlony's immunodiffusion test (Ouchterlony 1958). The patient underwent lung biopsy by thoracotomy.

One part of biopsy underwent routine paraffin embedding, sectioning and haematoxylin-eosin staining, the other part was

frozen and 4 µm thick cryostat sectioning and immunofluorescent staining was performed. Sections mounted onto glass slides were incubated in OKT3-, OKB7-, OKT4-, OKT8-, OKDR-FITC conjugates (Ortho-Diagnostics; dilution 1:10 in PBS), anti human IgG-, IgM-, IgA-FITC conjugates (Atlantic Antibodies, Scarborough; dilution 1:500 in PBS) and anti human IgG_1-, IgG_2-, IgG_3- and IgG_4-FITC conjugates (Pel Freeze dilution 1:100 in PBS) for 30 min at room temperature and rinsed afterwards, three times in PBS. For the detection of allergen specific antibodies on lung tissue, pigeon faecal extract, Cephalosporium and Pullularia antigens were conjugated to FITC; allergens were dialyzed in PBS (pH 7.2) overnight to remove preservatives such as sodium acid. Antigen-extracts were equibrilated with 0.05 mol Na_2HPO_4 to a pH 9.2. A stock solution with 50 µg FITC in 1 ml distilled water was prepared and 15 µg FITC was added per mg of antigen-extract and mixed at room temperature for two h in the dark. Afterwards antigen-conjugates were equibrilated with 0.1 mol Ka H_2PO_4 to pH 7.2. Conjugates were fractionated in a Sephadex G25 column and the F/P ratio – determined by light extinction at 280 nm and 495 nm – was 2.4 (Seelig 1983). Ouchterlony's immunodiffusion test was performed with FITC conjugated antigens in order to demonstrate the biological activity of the antigens with the patients serum-precipitating antibodies. Cryostat sections were then incubated 60 min with FITC conjugated antigen extracts, rinsed three times in PBS and evaluated in a Leitz photo-fluorescence microscope with FITC outfit.

Results

Light microscopic evaluation showed the typical pathological feature of HP, most prominently a patchy interstitial infiltrate extending widely into the parenchyma. The infiltrate consisted of histiocytes – sometimes with a foamy cytoplasm – lymphocytes, plasma cells, some neutrophils and occasional multinucleated giant cells (Fig. 1). The histiocytes occasionally formed sarcoid like non-caseating granulomas. There were no signs of vasculitis. In some areas a slight or moderate mural fibrosis was present.

Lymphocytic infiltrates consisted predominantly of OKT3 positive cells. We found less OKT4 positive helper cells than OKT8 positive suppressor cells and more than 20 percent of these T-lymphocytes reacted with OKDR antibodies. Deposits of IgG and IgM were found in the subendothelial position in blood vessels but immunoglobulins were also absorbed on alveolar macrophages (Fig. 2). Increased deposits of IgA were not detected by means of the indirect immunofluorescent test. The deposits of IgG in vessels belonged to IgG_1, IgG_2 and IgG_3 subclasses and the absorbed immunoglobulins on alveolar macrophages to all IgG subclasses. IgG_1 and IgG_3 showed the brightest staining. Immunofluorescent staining with FITC conjugated pigeon faecal extract showed a positive reaction in the same subendothelial position in vessels as the deposits of immunoglobulins. The same was true of alveolar macrophages (Fig. 3). No positive reaction was found between the lung tissue and antigens of Pullularia and Cephalosporium.

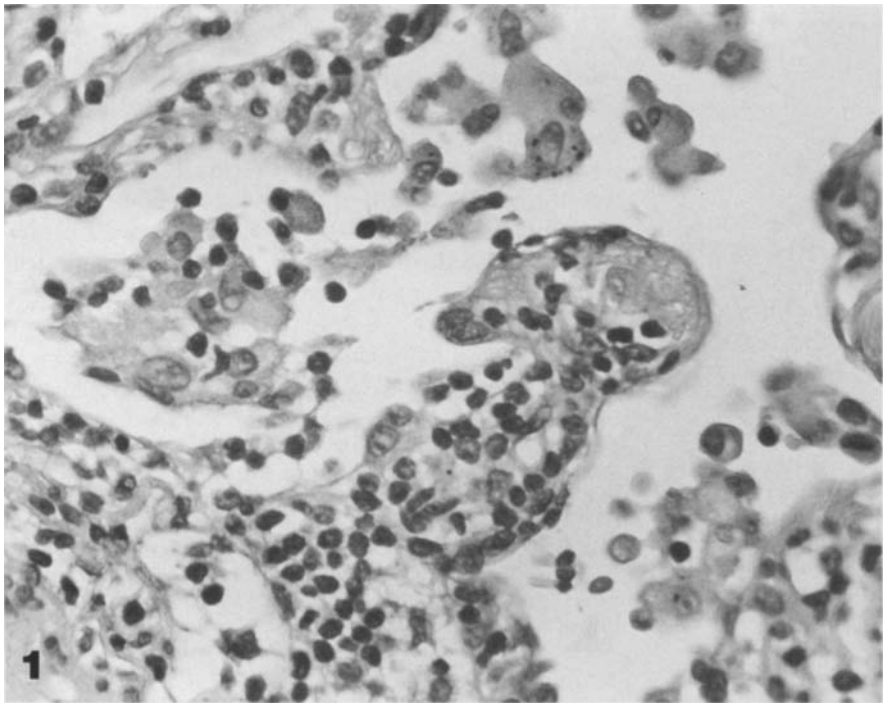

Fig. 1. Hypersensitivity pneumonitis showes interstitial infiltrates with lymphocytes, plasma cells and some histiocytes. Alveolar macrophages are encountered and tend to desquamate (×240)

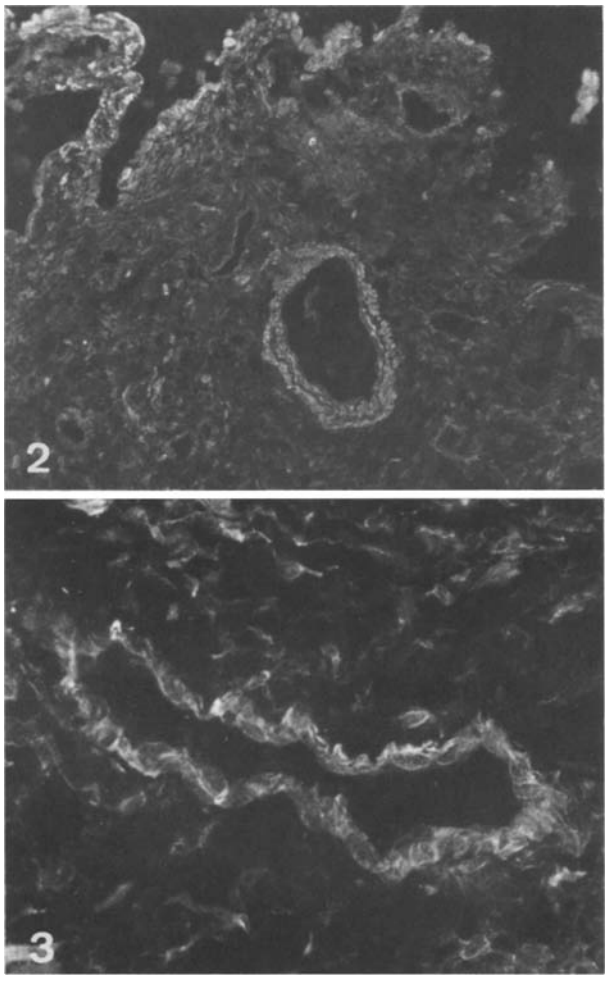

Fig. 2. Immunofluorescent test detected IgG antibodies in subendothelial positions in blood vessels and on alveolar cells (×80)

Fig. 3. Immunofluorescent test with pigeon faecal extract conjugated to FITC detected allergen specific antibodies in subendothelial positions in blood vessels (×160)

Discussion

Histological findings as alveolar cell desquamation, interstitial infiltrates with lymphocytes and plasma cells and formation of epitheloid cell-like, non-caseating granuloma indicate the diagnosis of HP, but there is no single morphological sign which is specific for HP. Immunohistochemical findings may confirm a suspected diagnosis of HP (Braun et al. 1986; Johnson et al. 1979; Salvaggio and Karr 1979).

Immunofluorescent staining detected IgG and IgM in the subendothelial position in blood vessels and on alveolar macrophages. The IgG deposits belonged to IgG_1 and IgG_3 subclasses which are well known to activate the classical pathway of the complement system. On alveolar macrophages all IgG-subclasses were present, perhaps due to a less specific defence mechanism against antigens. A predominance of suppressor cells, DR bearing lymphocytes and alveolar macrophages supported the hypothesis of a type IV-reaction in HP (Braun et al. 1986). Even with these immunological findings HP is not demonstrated unequivocally. The question arises whether HP in our patient was cryptogenetic or was caused by one, two or all of the antigens against which precipitating antibodies were found in serum (pigeon faecal extract, Cephalosporium, Pullularia antigens). However, lung tissue reacted only with pigeon faecal extract antigens. Furthermore, the immunoglobulin deposits in the blood vessels and the absorbed immunoglobulins on alveolar macrophages were characterized as antigen-specific.

Immunohistochemical investigations of immunoglobulin deposits and lymphocyte subpopulations are helpful diagnostic methods for the confirmation of HP. The characterisation of these immunoglobulins as allergen-specific antibodies in lung tissues may help to confirm the diagnosis and illustrate the aetiology in patients with HP.

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