

Asthma and extrinsic allergic alveolitis due to *Merulius lacrymans*

I. M. O'BRIEN,* J. BULL,* B. CREAMER,* R. SEPULVEDA,†
M. HARRIES, P. S. BURGE and J. PEPYS

Department of Clinical Immunology, Cardiothoracic Institute, and *St Thomas's Hospital, London, and †National Institute of Respiratory Diseases, University of Chile, Chile

(Received 16 May 1978; accepted for publication 30 May 1978)

Summary

A teacher with breathlessness of insidious onset developed acute symptoms on return home following his discharge from hospital. His flat was found to have extensive dry rot (*Merulius lacrymans*). Precipitins and specific IgE and IgG antibody against *M. lacrymans* were present and intracutaneous testing gave a typical dual skin reaction. Pulmonary physiology demonstrated airflow obstruction with a low DL_{CO} and K_{CO} , and a chest X-ray showed diffuse micronodular shadowing, maximal in the mid-zones. Inhalation challenge testing provoked a combined asthmatic reaction without a change in DL_{CO} . Rapid clinical recovery and more gradual radiographic and physiological improvement followed cessation of exposure to the antigen.

Introduction

Extrinsic allergic alveolitis due to the inhalation of organic dusts, such as fungal spores and thermophilic and heterologous serum protein, is well recognized (Pepys, 1969; Carroll *et al.*, 1975) and occurs mainly in non-atopic subjects. In some subjects, however, a type I hypersensitivity reaction occurs, giving an immediate asthmatic reaction followed by a late reaction in the gas-exchanging part of the lung (Riddle *et al.*, 1968; Lunn & Hughes, 1967).

Dry rot was recognized in Biblical times as 'The plague or leprosy in the house' (Leviticus). The causative fungus, *Merulius lacrymans*, belongs to the order Aphyllophorales, one of the three constituting the class Basidiomycetes. Airborne basidiospores ($8.0-10 \times 5.0-6.0 \mu m$) infect wood with a water content of 20-25% and mycelia develop and extend in sheets over timber and adjacent brickwork. Fruiting bodies develop as flat, fleshy surface growths and immense numbers of rusty red basidiospores are produced, which may form deposits visible to the naked eye. *M. lacrymans* is a

Correspondence: Professor J. Pepys, Department of Clinical Immunology, Institute of Diseases of the Chest, Brompton, London S.W.3.

fungus of buildings and is rarely found away from areas of human habitation. It is widely distributed in the cool temperate zones of Northern Europe.

Asthma due to the spores is established (Frankland & Hay, 1951; Herxheimer, Hyde & Williams, 1966, 1969) and inhalation testing in the present case provoked a combined asthmatic reaction. There was, however, other evidence of allergic alveolitis which has not previously been reported in relation to *M. lacrymans*.

Case report

A 32-year-old male, primary school teacher, with no previous history of respiratory illness, developed a persistent, unproductive cough in December 1976. Over the succeeding 3 months he became progressively dyspnoeic, developed malaise, intermittent arthralgia and fever, and lost 6.5 kg in weight. Treatment with tetracycline and amoxycillin produced no improvement, and by March 1977 he had become dyspnoeic on slight exertion and was admitted to hospital. On examination he was dyspnoeic at rest and there were inspiratory crackles at the lung bases. A chest X-ray (Fig. 1a and b) showed diffuse, micronodular and linear shadowing, most marked in the midzones, and septal lines. Tomography showed no evidence of hilar adenopathy. Routine haematology and eosinophil count were normal and the ESR was 47 mm in 1 hr. Agglutination titres to common viruses and *Mycoplasma pneumoniae* were normal and showed no rise after a 10 day interval. He improved spontaneously and after 3 days the basal crackles had disappeared and his exercise tolerance was greatly improved. He was discharged after 10 days with an unchanged chest X-ray.

Following discharge he returned home and after 4 hr his cough recurred and he became progressively dyspnoeic. He was readmitted the following day and referred to the Brompton hospital with a provisional diagnosis of extrinsic allergic alveolitis.

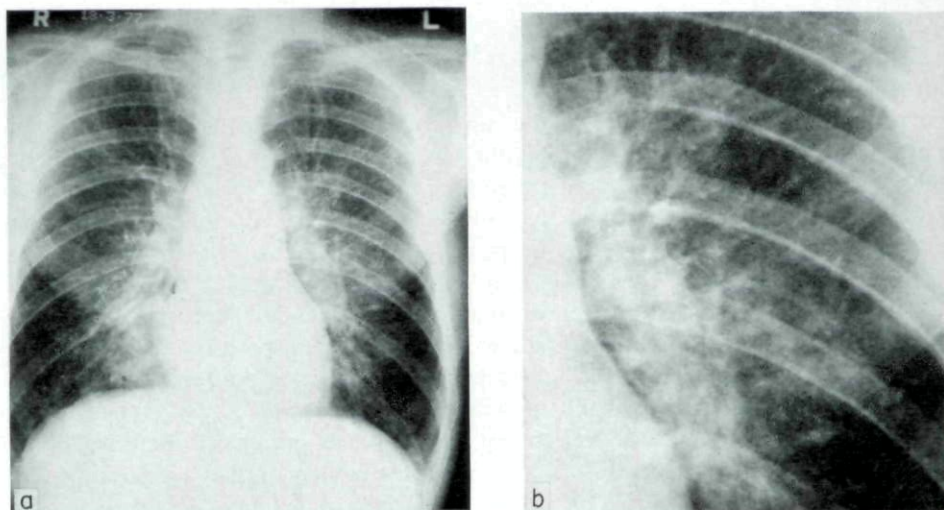


Fig. 1. (a) and (b) Chest radiograph showing micronodular shadowing, 18.3.77.

Investigations

Skin tests

Prick tests were made with the following common allergen extracts (Bencard): *Alternaria* species, *Aspergillus fumigatus* and *A. terreus*, *Cladosporium herbarum*, *Merulius lacrymans*, *Sporobolomyces roseus*, yeast, *Candida albicans*, grass, shrub, tree and nettle pollens, milk, egg, wheat, fish, nuts, cat, dog, feathers, horse, house dust, *Dermatophagoides farinae* and *D. pteronyssinus*.

Intracutaneous tests were made with 0.02 ml of extracts of *M. lacrymans* (10 mg/ml) and *A. fumigatus* (10 mg/ml).

Precipitin tests

These were made by agar gel double diffusion (Longbottom & Pepys, 1964) to *A. fumigatus*, *C. albicans* and to two freeze-dried extracts of *M. lacrymans* (30 mg/ml); one prepared in 1968 and the other in 1977 from fungus collected from the subject's home (and confirmed by botanists as *M. lacrymans*). Tests were also made on the sera of twenty-seven subjects with unrelated lung disease with the 1977 antigen and on ten with the 1968 antigen. Tests by immunoelectrophoresis to the same *M. lacrymans* extracts (60 mg/ml) (Grabar, 1960) were made on the subject's serum.

Enzyme-linked immunosorbent assays (ELISA) for specific IgG and IgE

The indirect microplate ELISA method for detection and measurement of specific IgG to *M. lacrymans* was performed by an adaptation of the method described by Voller & Bidwell (1976). Wells of polystyrene microhaemagglutination plates were sensitized by pressure absorption with *M. lacrymans* (50 mg/ml) and incubated with the test serum. Rabbit anti-human IgG conjugated with alkaline phosphatase (Sigma type VII) by the glutaraldehyde method (Engvall & Perlmann, 1972) was added, followed by the enzyme substrate, and the colour change was assessed using a 405 nm Vitatron spectrophotometer. The colour change in absorbance units is proportional to the specific IgG antibody concentration of the test serum.

A modification of the above method was used for the determination of specific IgE. Following sensitization of the polystyrene plates and incubation with the test serum, rabbit anti-human IgE was added and left overnight. Sheep anti-rabbit serum conjugated with alkaline phosphatase was added followed by enzyme substrate. The colour change, assessed spectrophotometrically, was proportional to the specific IgE antibody concentration of the test serum.

The patient's serum was tested for specific IgE and IgG on admission and 7 months later after clinical, radiological and physiological recovery. Tests for IgG were performed on controls with positive precipitins to *M. lacrymans*, and for IgG and IgE on controls with negative precipitins.

Inhalation challenge tests

Aerosol challenge was performed by passing oxygen at 8 l/min through allergen in a Wright's nebulizer, the subject inhaling through a face mask from a rebreathing bag. The same apparatus was used for each test and only one test was performed on any one day.

The following challenges were performed on separate days: a control test with

Coca's solution; *M. lacrymans* at 0.1 mg/ml, 1.0 mg/ml and 10 mg/ml (1968 antigen), and 10 mg/ml (1977 antigen).

FEV₁ and FVC were recorded using a dry-wedge spirometer (Vitalograph) prior to and following challenge at 5 min intervals for the first 30 min, at 10 min intervals for the next 30 min, at 30 min intervals for the next 60 min and then at hourly intervals for at least 8 hr. DL_{CO} was measured using the single breath technique before and 6 hr after challenge. The temperature was recorded hourly.

Pulmonary function tests

FEV₁ and FVC were measured using a wet spirometer, lung volumes by whole body plethysmography and diffusing capacity of the lung for carbon monoxide (DL_{CO}) by the single breath technique.

Blood tests

Measurements of haemoglobin, total white cell count and wet eosinophils were made on numerous occasions. Tests were also made for immunoglobulins, Rose-Waaler titre, ANF and other non-organ-specific antibodies and Clq inactivator (esterase inhibitor).

Other tests

Fibreoptic bronchoscopy, transbronchial lung biopsy and serial chest X-rays were performed.

Results

Skin tests

Prick testing gave a single weak positive reaction to grass pollen.

Prick testing with *M. lacrymans* was negative, but an intracutaneous test produced a weal and flare at 15 min, which subsided by 1 hr, and was followed by a late, oedematous reaction measuring 7.5 × 4.5 cm at 6 hr, which subsided by 36 hr.

An intracutaneous test with *A. fumigatus* was negative.

Precipitin tests

Agar gel double diffusion testing gave a single precipitin line to both 1968 and 1977 antigens. Eight of twenty-seven controls gave precipitin reactions to the 1977 antigen, but none were positive to the 1968 antigen. Immunoelectrophoresis gave a single precipitin line.

Agar gel double diffusion tests with *A. fumigatus* and *C. albicans* were negative.

ELISA for specific IgG and IgE

The results for the patient and for two controls are shown in Fig. 2, with higher values for IgG and IgE in the May as compared with the December 1977 serum samples. The precipitin-positive control showed the presence of IgG but not IgE antibody, and a precipitin-negative control gave low values for both IgG and IgE.

Inhalation challenge tests (Table 1)

A combined asthmatic reaction was provoked by both 1968 (Fig. 3) and 1977 antigens

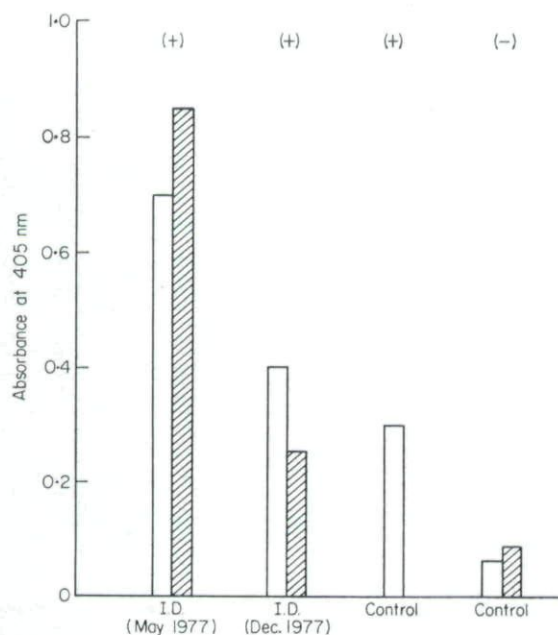


Fig. 2. Indirect microplate ELISA method for measurement of anti-*M. lacrymans* antibody. Antigen was *M. lacrymans* (50 µg/ml). Pluses and minuses in parentheses show presence of precipitins. (□) IgG; (▨) IgE.

Table 1. Inhalation challenge tests

Test material	Concentration (mg/ml)	Duration (min)	Result
Coca's solution		5	Negative
1968 antigen			
<i>M. lacrymans</i>	0.1	5	Negative
<i>M. lacrymans</i>	1.0	5	Immediate asthmatic reaction
<i>M. lacrymans</i>	10	3	Immediate asthmatic reaction
<i>M. lacrymans</i>	10	15	Dual asthmatic reaction
1977 antigen			
<i>M. lacrymans</i>	10	5	Dual asthmatic reaction

at a concentration of 10 mg/ml, but there was no significant change in DL_{CO}, temperature or white cell count.

Blood tests

Haematology and wet eosinophils were normal; the autoantibody test was negative; the Rose-Waaler titre was positive at 1/16 dilution; Clq inactivator (esterase inhibitor) normal. Immunoglobulins were normal, with the exception of IgA at 307 iu (normal range 75-268 iu).

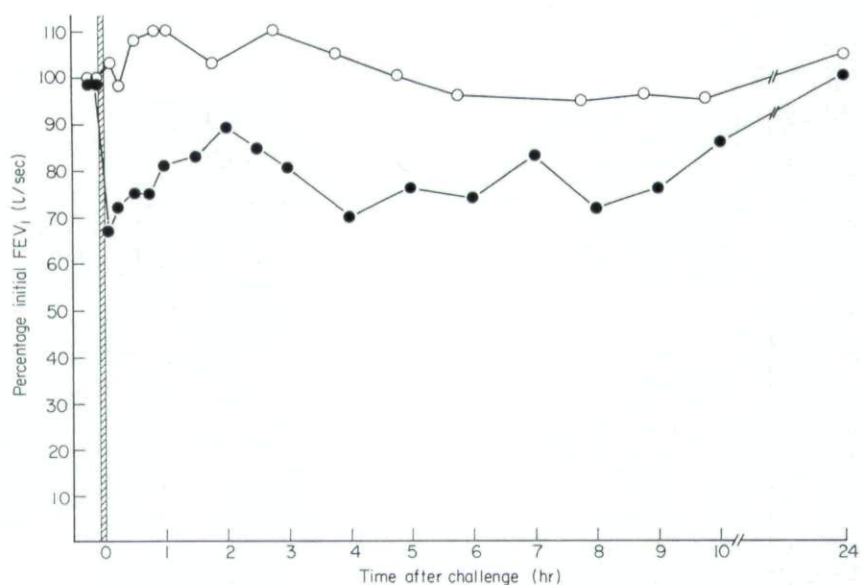


Fig. 3. Bronchial provocation test with *Merulius lacrymans*. (○) Coca's solution; (●) *M. lacrymans* (10 mg/ml).

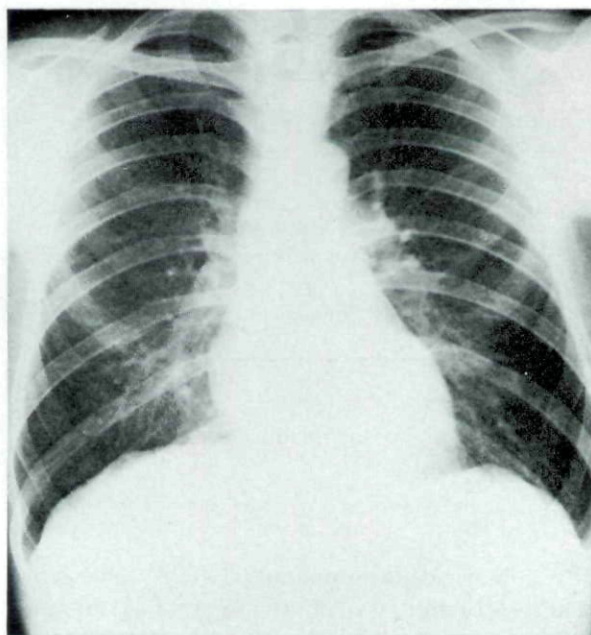


Fig. 4. Clearing on chest radiograph, 7.12.77.

Table 2. Pulmonary function tests showing gradual improvement after cessation of exposure

Test	Predicted	7.4.77	15.4.77	18.5.77	29.6.77	31.8.77	7.12.77
FEV ₁ (ml)	3180-4310	2170	2240	2880	3120	3480	3330
FVC (ml)	3850-5210	4620	4600	5150	5140	5570	5370
FEV ₁ /FVC (%)	78-86	47	49	56	61	62	62
TLC (ml)	5060-6850	6470	6950	6760	6960	6240	7380
RV (ml)	1430-1930	2570	2850	2310	2090	1160	2000
FRC (ml)	—	4470	4400	4210	3890	2840	4430
VA (ml)	5060-6850	5610	5520	6120	5990	5960	6160
VC (ml)	3850-5210	3900	4100	4450	4870	5080	5370
AWR (kPa/l/sec)	<0.2	0.00	0.00	0.00	0.00	0.00	0.00
SGAW (l/kPa/sec)	1.3-3.6	>3.6	>3.6	>3.6	>3.6	>3.6	>3.6
DL _{LO} (mmol/min)	8.92-12.07	6.55	7.35	7.84	8.99	8.54	10.52
K _{CO} (mmol/min/kPa/l)	1.52-2.05	1.17	1.33	1.28	1.50	1.43	1.71

Other investigations

Fibreoptic bronchoscopy was normal to the subsegmental level. Transbronchial lung biopsy provided a very small piece of tissue, histology of which showed a light, diffuse, interstitial infiltrate by lymphocytes, with four neutrophils and collections of histiocytes in two sites. Although suggestive, it was considered too small to be diagnostic of extrinsic allergic alveolitis.

Serial chest X-rays showed gradual resolution of the micronodular shadowing, following cessation of exposure by moving house, but normality was not reached until December 1977 (Fig. 4).

Table 2 shows the gradual improvement in spirometry, DL_{CO} and K_{CO} following cessation of exposure. After 8 months the DL_{CO} and K_{CO} were normal, but there was still evidence of mild airflow obstruction, although the FEV₁ and FVC had returned to the predicted range.

Discussion

The insidious onset of cough, dyspnoea, malaise, arthralgia and weight loss associated with the radiographic appearances, and the occurrence of acute symptoms on re-exposure to the antigen, are consistent with the subacute form of extrinsic allergic alveolitis. This pattern is most commonly seen in budgerigar fanciers, and attributed to regular exposure to the antigen. The rapid clinical recovery and more gradual improvement in the radiographic appearance and respiratory physiology after cessation of exposure are again consistent with this diagnosis.

The dual skin reaction and the presence of specific IgE and IgG to *M. lacrymans* suggest the involvement of both type I and III hypersensitivity reactions. The fall in specific IgE and IgG following cessation of exposure correlates with the clinical, radiological and physiological improvement.

The presence of precipitins in some of the controls as well as in the patient appears similar to the presence of precipitins to *M. faeni* in farm workers, indicating exposure and not necessarily associated disease.

The combined asthmatic reaction elicited by inhalation testing is comparable with that seen in allergic bronchopulmonary aspergillosis (Pepys, 1969). The failure to

provoke a peripheral lung reaction might be explained by the necessarily very limited challenge in the presence of airflow obstruction. Clinical exposure to fungal spores capable of reaching alveolar level could elicit a reaction in the gas-changing part of the lung.

In the immunopathogenesis of this patient's disease, both types I and III hypersensitivity reactions to *M. lacrymans* appear to be involved, as in the disease seen in malt workers due to the spores of *A. clavatus*.

References

- CARROLL, K.B., PEPYS, J., LONGBOTTOM, J.L., HUGHES, D.T.D. & BENSON, H.G. (1975) Extrinsic allergic alveolitis due to rat serum proteins. *Clinical Allergy*, **5**, 443.
- ENGVAL, E. & PERLMANN, P. (1971) Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of IgG. *Immunochemistry*, **8**, 871.
- FRANKLAND, A.W. & HAY, M.J. (1951) Dry rot as a cause of allergic complaints. *Acta allergologica*, **4**, 186.
- GRABER, P. (1960) *Analyses Immunoelectrophoretique* (eds P. Graber and P. Burtin), p. 28. Masson, Paris.
- HERXHEIMER, H., HYDE, H.A. & WILLIAMS, D.A. (1966) Allergic asthma caused by fungal spores. *Lancet*, **i**, 572.
- HERXHEIMER, H., HYDE, H.A. & WILLIAMS, D.A. (1969) Allergic asthma caused by basidiospores. *Lancet*, **ii**, 131.
- LEVITICUS. *Holy Bible*, Chap. 14, verses 33-57.
- LONGBOTTOM, J.L. & PEPYS, J. (1964) Pulmonary aspergillosis: diagnosis and immunological significance of antigens and C-reactive substance in *Aspergillus fumigatus*. *Journal of Pathology and Bacteriology*, **88**, 141.
- LUNN, J.A. & HUGHES, D.T.D. (1947) Pulmonary hypersensitivity to grain weevil. *British Journal of Industrial Medicine*, **24**, 158.
- PEPYS, J. (1969) Hypersensitivity disease due to fungi and organic dusts. *Monographs in Allergy*. Karger, Basel.
- RIDDLE, H.F., CHANNELL, S., BLYTH, W., WEIR, D.M., LLOYD, M., AMOS, W.M.G. & GRANT, I.W.B. (1968) Allergic alveolitis in a malt worker. *Thorax*, **23**, 271.
- VOLLER, A. & BIDWELL, D.E. (1975) Enzyme immunoassay in diagnostic method. Theory and practice. *World Health Bulletin*, **53**, 156.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.