PIGEON FANCIERS LUNG

A study of the clinical, lung function and immunological responses among pigeon fanciers.

by

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JANUARY 2005

ACKNOWLEDGMENTS

I would like to personally thank Dr Charles McSharry for his supervision, advice, support and endless encouragement during all stages of this thesis. I am indebted to him and many others who made this thesis possible.

I would also like to thank Iona Fraser for her assistance in the lab work.

It is pleasure to record my thanks to Dr Phillip Lynch for his advice and help in organising the study done with the North West Federation Fanciers Club.

The success of this thesis was largely attributed to the enthusiastic participation of the many pigeon fanciers. I would also like to thank The British Pigeon Fanciers Medical Research Team for their financial support and their involvement has been vital in the data collection for this thesis.

It is a pleasure also to thank Dr Robert Milroy for all his support and encouragement.

I am personally grateful to Dr Gavin Boyd for the all his guidance, kindness and advice during the research.

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SUMMARY OF THESIS

Pigeon Fanciers Lung (PFL) is one of the commonest causes of Extrinsic Allergic Alveolitis (EAA) or Hypersensitivity Pneumonitis (HP). It is induced by repeated inhalation of antigens from pigeons in a sensitised individual. Since the majority of fanciers do not develop the disease despite being exposed to similar amount of antigen exposure, the host's susceptibility factors including constitutional and environmental factors and the combination of these factors are prerequisite to the development of disease. Among these predisposing factors, it has been proposed that certain genetic susceptibility such as alleles of the major histocompatibility complex may increase an individual's susceptibility to develop the disease. Other environmental factors such as cigarette smoking and amount of antigen exposure may also play a role.

PFL is characterised by inflammation of the lung parenchyma but it also involves small and large airways. Apart from respiratory symptoms, fanciers with PFL also develop systemic symptoms such as fever and myalgia that usually occurs 4-8 hours after antigen exposure and can last until 24 hours. The sequence of immunopathological events that contribute to the development of PFL is unresolved. Evidence supports the role of immune complexes with the exuberant antibody response and the delay in the onset of symptoms. T cell mediated response also plays a vital role with the appearance of granuloma. It is likely that these 2 processes occur simultaneously and complement each other as the immune response progresses.

In view of the different clinical presentations and dynamic nature of the disease, the disease can be divided into 3 groups consisting of acute progressive, acute intermittent non progressive and chronic disease. Fanciers with an antibody response but without symptoms should be regarded as having subclinical disease as there is evidence of

ongoing immune and inflammatory response and they are at risk of progressing to clinical disease.

This thesis examines the immunological response and its correlation with the clinical status and other factors that may influence this response amongst pigeon fanciers. The clinical material was collected from a group of pigeon fanciers from the North West Federation Fanciers and also from pigeon fanciers attending pigeon shows mainly in Blackpool.

Most of the studies in PFL are done on patients attending clinics or volunteers attending a pigeon show and there is a lack of epidemiological data. In a cohort of 41 pigeon fanciers studied over 5 years, there was deterioration in lung function measured by spirometry. These changes were associated with T lymphocytes abnormalities but there was no change in the clinical status over the 5 year period.

Part of this study examined a large population of pigeon fanciers and found that the majority of fanciers showed evidence of sensitisation. Specific IgG antibody to avian antigen is a good indicator for antigen exposure and is an important part of the diagnostic workup. The IgG antibody response against avian antigen was quantified by ELISA in a population of pigeon fanciers and measured against several constitutional and environmental factors that may affect the antibody response. The ability to produce an antibody response seems to increase with age but the response is not influenced by the amount of antigen exposure. Therefore the antibody response appeared to be determined by the individual's own immunological responsiveness rather than the amount of antigen exposure. Cigarette smoking inhibited IgG antibody production and this effect appears to be reversible as the antibody levels in ex smokers is similar to non-smokers. Fanciers with the highest amount of antibody production tend to be the most symptomatic. There was an association between increase incidences of PFL with progressively higher antibody production. The incidence of probable PFL was 42% in fanciers with a IgG antibody level of above 30ug/ml. There was also no proven correlation between IgG antibody response and airflow limitation among the pigeon fanciers. Some fanciers

without any evidence of sensitisation had respiratory symptoms similar to PFL that may be due ornithosis or to many other antigens and irritant dusts that has been shown to be present in the pigeon loft.

Animal studies have supported the role of cytokines in the pathogenesis of HP. The initial acute symptoms in PFL suggest the involvement of proinflammatory cytokines and the regulatory cytokines are involved in polarising the lymphocytes primarily towards a Th1 type response. In this thesis the role of proinflammatory and regulatory cytokines were studied. In fanciers with symptoms suggestive of PFL there was an increase in proinflammatory cytokines TNFα, IL-1β, IL-6, IL-8 and regulatory cytokines IL-18. These antigen induced cytokine levels correlated with the avian antigen specific serum antibody level. There was no difference in the constitutive or endotoxin inducible level of this cytokines suggesting that the cytokine production mediated by immune complexes was quantitatively and qualitatively different from cytokine profile produced by endotoxins and may predispose to disease. Studies have shown that T cell responses produce different patterns of cytokine production. The ability to produce high or low amount of cytokines and the interaction between them may determine the susceptibility of the individual as these cytokines may provide protection or stimulate pathological immune responses in the individual.

T cell lymphocyte abnormalities have been suggested to be involved in the pathogenesis of airflow limitation in patients with COPD. This thesis examined the association between FEV1 and CD8+ T cell lymphocyte and found that fanciers with symptoms suggestive of PFL had a significant increase in CD8+ cells and there was an association between FEV1 and CD8+ lymphocytes in peripheral blood among the pigeon fanciers.

This study emphasises the complexity and the dynamic nature of this disease. The variation in each individual's immune responsiveness towards avian antigen and the various different immunological mechanisms involved in the disease process is highlighted.

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1. INTRODUCTION

There are more than 80,000 registered pigeon fanciers in the UK and can be found in almost all countries in the world. Although the hobby is concentrated around previous mining and industrial areas it is practiced throughout the country and not confined to any particular socioeconomic group.

Pigeon Fanciers Lung (PFL) is a form of extrinsic allergic alveolitis (EAA)/ hypersensitivity pneumonitis (HP) caused by repeated inhalation of pigeon antigens in sensitised subjects. HP is caused by a wide variety of antigens (Table 1). The antigens include bacteria, organic materials, fungal and chemicals. The exposure to these antigens occurs in various settings including occupational, hobbies and the environment. Although the antigens are specific for each of the syndromes the clinical presentation of the different syndromes is similar suggesting the subsequent hypersensitivity reaction is homogenous.

The pathogenesis of PFL is thought to be as a result of type 3 hypersensitivity reaction/ immune complex mediated response with the presence of avian antigen specific antibodies in the serum and BAL of affected individuals. There is also a delay in the development of symptoms and the timing and histological findings are similar with an Arthus like skin reaction. However the appearance of granuloma in the lungs and asymptomatic individuals with a positive antibody levels suggests involvement of type 4 hypersensitivity reaction/ cell mediated immune response as well. The two may occur either simultaneously or at different stages of diseases where the immune complex-mediated immune response induces the acute lung injury via complement-dependent neutrophils and the T cell mediated immune responses induces granulamatous reaction in the later stages.

Of the various HP syndromes, Farmer's Lung and PFL are the commonest form that has been studied. There has been a decrease in the incidence of Farmer's Lung due to the changes in the farming practice. PFL is now the most commonly HP that presents to the clinic and studied. It has considerable research potential as a model for lung inflammation (Table 2). Previous studies are based on patients presenting to the clinic and volunteer pigeon fanciers attending pigeon shows. The majority of pigeon fanciers who present themselves to clinics or participate in research do have evidence of sensitisation but only a minority will develop the disease. There is a lack of epidemiological study of this disease and no good data on disease prevalence.

Syndrome	Antigen
Microbes / Fungal	
Farmer's Lung	Thermophilic actinomycetes
	Saccharopolyspora rectivirgula
	(Micropolyspora Faeni)
	Thermoactinomyces vulgaris
Bagassosis	Thermoactinomyces sacchari
Mushroom Workers Lung	Thermoactinomycete sp
	Agarieus hortensis spores
Humidified Lung	Micropolyspora faeni
Suberosis	Penicillium frequentans
Malt Workers Lung	Aspergillus clavatus
Woodworkers Lung	Penicillium chrysogenum
Cheese Washers Lung	Penicillium casei
Maple Bark Strippers Lung	Cryptostroma corticale
Paprika Splitters Lung	Mucor stolanifer
Hot Tub Lung	Mycobacterium avium intracellulare
Summer type HP	Trichosporon cutaneum
Sax Lung	Candida albicans
Animals	
Pigeon fanciers lung	Avian proteins
Animal handlers lung	Laboratory animals
Fish meal workers lung	Fish meal extract
Rat handlers lung	Rat serum proteins
Chemicals	
Isocyanates	Paints, plastics
Anhydrides	Plastics

Table 1. Causative agents causing EAA/HP

2. CLINICAL

i) Symptoms

PFL is a complex dynamic clinical syndrome where clinical expression depends on the external antigens interacting with the host's immune system. This is complicated by self-regulatory measures performed by fanciers to reduce antigen exposure. There are many individuals with a positive antibody reaction but remain asymptomatic.

HP traditionally has been described as occurring in a state of acute, subacute and chronic form. The symptoms of acute phase (Table 3) tend to occur 4-8 hours after exposure of offending antigen and persist up to 12 hours. Symptoms usually resolve within 24 hours unlike a viral infection.

Given the dynamic nature of the disease and evolution of different clinical patterns over time an alternative classification system has been proposed (1,2).

1. Acute progressive – patients experience severe symptoms after antigen exposure and symptoms progress after each subsequent exposure such that patient often recognises the problem and usually try to modify their antigen exposure or stop antigen exposure completely. Clinically they may be pyrexial and have bilateral crackles on chest auscultation with radiological changes on chest radiograph. They may require admission to hospital for treatment because of respiratory compromise or the severity of symptoms.
2. Acute intermittent non–progressive – this is the commonest form of disease in this British pigeon fanciers population. Fanciers develop similar symptoms as acute progressive disease but less intense and they feel well in between episodes. Many of these subjects recognise and indeed anticipate symptomatic episodes after certain activities involving greater than normal exposure to their birds (Table 4). They continue to have exposure to the antigen and paradoxically symptoms may be less

severe with recurrent exposure. Their long term clinical picture appears stable with no deterioration in clinical status or lung function. Bourke et al studied the longitudinal course of PFL in a group of pigeon fanciers 10 years after they presented with the acute form of diagnosis and despite continued antigen exposure the symptoms related to pigeon antigens had improved in the majority of patients and lung function remained stable (3). Studies in animal models have also shown that continued antigen challenge results in waning of the pulmonary inflammatory response rather than progression of disease (5,6).

3. Chronic progressive disease- this may occur in patients following recurrent acute episodes or may occur insidiously without any history of acute episodes (7). In the insidious group, most patients have few acute symptoms if any and the symptoms may be non specific such as worsening dyspnoea on exertion, anorexia, weight loss, malaise and cough. There is often a delay in making the diagnosis and this is the form of the disease in which the patient presents with permanent disability such as chronic shortness of breath, pulmonary fibrosis and respiratory failure. It is often indistinguishable from other forms of fibrotic lung disease. The history of avian antigen exposure may be the only clue in the diagnosis and on occasions subsequent avoidance of antigen may not reverse the disease (8).
4. Subclinical disease – there is a large overlap in antibody response between symptomatic and asymptomatic pigeon fanciers. Fanciers with antibody response but without the symptom warnings and subsequent modification of antigen exposure, may progress to the chronic, non reversible form. Although these patients may not have obvious clinical symptoms there is evidence that there is ongoing immunological and inflammatory response from the host.

1. Population at risk identified
2. Obvious source of antigen
3. Antigen available
4. Exposure indices can be measured
5. Antibody response can be measured
6. Relationship between symptoms and immune response can be establish

Table 2. Model for lung inflammation

Systemic	**Respiratory**
Fever	Cough
Sweating	Breathlessness
Myalgia	Wheeze
Aching joints	Chest tightness
Headaches	
Flu like symptoms	

Table 3. Respiratory and systemic symptoms of PFL.

Early in season when young birds are taken in a car to a distant location to train them
During race times (May to August) when fanciers spend many hours in loft
When lofts are cleaned out
When the birds moult and shed their feathers (September to November)(4)

Table 4. Seasonal presentation of pigeon fanciers lung

Surveys of pigeon fanciers have also revealed a variety of other symptoms (Table 5) that suggests there are many sources of antigenic sensitisation, infection or inflammation associated with pigeons. Although EAA/HP is the commonest, many other diseases have similar symptoms and presentations.

There is a high prevalence of chronic bronchitis among non smoking pigeon fanciers and these increases with higher antibody levels. 8.4% of pigeon fanciers surveyed had chronic bronchitis as their only manifestation of pigeon symptomatology (9,10). The increase in incidence of asthma and rhinitis in pigeon fanciers may be due to other antigens such as bird feather mites or storage dust mites. Some fanciers complain of immediate symptoms after exposure such as cough, wheeze, sneezing and watering of eyes. This may be due to larger airborne particles, which trigger IgE mediated hypersensitivity responses that gain access to the large airways, nasal mucosa and the conjunctivae. Human ornithosis is due to cross infection from pigeons, which harbour many organisms that can infect humans (Table 6). Evidence of sensitisation to respiratory viruses has been reported but explained as an inflammation associated anamnestic response (11). Despite maintaining healthy birds, serological sensitisation to chlamydial psitaci among pigeon breeders is common (12,13).

It is not known what factors determines the initial presentation and clinical course of the disease. The development of clinical disease depends on the antigen exposure, host's susceptibility and the resulting level of dysregulation of the cellular and immune response over time. Table 7 shows the constitutional and environmental factors that may affect the susceptibility of an individual to the antigen and the development of the disease.

The level and intensity of antigen exposure may be an important factor in the development of disease. In Mexico the disease tends to affect women and tends to favour the development of the chronic form of disease and development of pulmonary fibrosis (22). The practice of keeping small number of pigeons at home as pets, providing prolonged low grade antigen exposure may explain this. This is in contrast to British

EAA/HP - commonest
Chronic bronchitis (9)
Bronchiolitis (46)
Asthma and rhinitis (107-109)
Human ornithosis

Table 5. Diseases associated with pigeons

Bacteria	Chlamydia sp, Mycoplasma, M avium, E coli
Fungi	C. Albicans, aspergillus
Viruses	Adenovirus, Influenza
Parasites	Trichomonads
Mites	Diplaegidia columbae

Table 6. Pigeon associated organisms

Constitutional	Environmental
Age	Smoking (15)
Genetic factors – HLA phenotype (14)	Antigen exposure
Cytokines (18,19)	Loft ventilation (16,17)
	Infection-viral, bacterial (20,21)

Table 7. Constitutional and environmental factors affecting the susceptibility to develop the disease

pigeon fanciers, where they tend to be exposed to intermittent high levels of avian antigen when in their pigeon lofts where the commonest form of the disease is acute intermittent non-progressive. There is a seasonal variation in antibody levels among pigeon fanciers depending on the time spent on training their birds during the racing season and the increase in the amount of antigen exposure when the pigeon moult and shed their feathers (4). Air sampling studies have shown a significant increase in the amount of respirable dust level and antigen content during the autumn moult corresponding to the increase in expression of clinical symptoms among pigeon fanciers during that period (17)

Viral infections are known to modulate the immune response. Some patients report that though they had been exposed for years, the symptoms only appeared after an acute respiratory infection. Mice infected with Sendai virus and simultaneously sensitised with antigens develop an enhanced response to the antigen that persists even after the viral infection has resolved. Viral infections can increase the antigen presenting capacity of alveolar macrophages by increasing the MHC class 1 and 11 molecule expression (20). It contributes to the accumulation and proliferation of lymphocytes by increasing the expression of adhesion molecules ICAM-1 and VCAM-1 and major histocompatibility antigens (23). Viruses also induce pro-inflammatory cytokines and production of interferon gamma and IL-8 favouring the proliferation of TH-1 lymphocytes that are putatively associated with HP.

The genetic differences between individuals must be important in determining the clinical outcome of exposure. The genetic polymorphism for production of high or low amounts of inflammatory cytokines may be involved. Pigeon fanciers with HP has been shown to have an increase of the frequency of HLA –DRB1*1305, HLA-DRQB1*0501 and TNF-α (308) promoter which is associated with high production of TNF-α (14). This study looked at the role of pro inflammatory and regulatory cytokines in determining the susceptibility to the disease.

ii) Investigations

There is no single test specific for PFL (Table 8). Physical examination is often unhelpful in establishing the diagnosis but the presence of crackles on auscultation of the chest an fever should alert the clinician of the possibility of the disease. Routine blood tests are usually not helpful. There may be an increase in the erythrocyte sedimentation rate, C-reactive protein, leucocyte count and gammaglobulins. The rheumatoid factor is often positive although the exact cause of this is unknown (24). The avian IgG antibody will be increased in the majority of individuals exposed to avian antigen and is a good marker for monitoring ongoing inflammatory response. However there are cases of reported negative antibody response in patients although this may be because of the insensitivity of the test.

The radiological findings on chest radiograph and high resolution computed tomography (HRCT) of the chest could provide important clues towards the diagnosis of PFL. The radiographic findings will correlate with the stage of the disease. In the acute stage the CXR findings include diffuse ground-glass opacification and fine nodular or reticulonodular pattern, often involving the lower zones. This reticulonodular pattern becomes more prominent with ongoing inflammation. In the chronic disease, the findings include upper lobe fibrosis, reticular opacity, volume loss and honeycombing (25).
HRCT is significantly more sensitive than chest radiograph for detecting PFL (26). The findings on HRCT have been correlated with histological findings and lung function abnormalities in HP (27,28) In the acute stage, centrilobular nodules, which are poorly defined and smaller than 5mm in diameter, are found predominantly in the mid and lower zones (29,30). Centrilobular nodules probably represent the peribronchiolar lymphatic infiltration that is seen in histopathology (28). Ground glass shadowing, which is thought to represent either active interstitial inflammation or fine fibrosis (31), is also seen in the acute stage (32) and with the presence of centrilobular nodules these changes are highly suggestive of HP (26). Both these changes tend to resolve with cessation of antigen exposure (27). It may also show a mosaic pattern and evidence of air trapping (28,33). In the chronic stage, irregular opacities, traction bronchiectasis, loss of lung volume and honeycombing usually indicates irreversible fibrosis (26).

Gallium scan and TcDTPA lung clearance shows changes due to lung inflammation but is not routinely performed to aid the diagnosis. Lung function tests are mandatory to assess any impairment to the lungs and will be discussed later. Provocative testing by inhalation challenge is rarely required to be performed to make the diagnosis.

If the above investigations are still not sufficient to confirm the diagnosis the usual next step is to obtain bronchial alveolar lavage (BAL) fluid and diagnostic lung tissue.
BAL is a minimally invasive and safe technique that has become a standard diagnostic procedure for the majority of patients with interstitial lung disease. The BAL findings in interstitial pulmonary fibrosis show a predominant T helper 2 cytokine profile, whereas the BAL findings in sarcoidosis and HP are characterised by a dominant T helper 1 profile. The BAL findings are dependent on the timing of last antigen exposure, stage of the disease and smoking habit. In the acute stage there is an influx of neutrophils (34) and after 24 hours there will be a predominance of lymphocytosis with a relative predominance of CD8+ T cells, interferon gamma and TNF receptors 1 and 2 (35-37). The lymphocytosis in BAL correlates with the severity of alveolitis (38) suggesting the selective recruitment and retention of these lymphocytes are vital. There is a correlation between tissue lung biopsy levels of CCL18, which is putatively involved in naive T cell recruitment, and the number of lymphocytes recovered from the BAL fluid (39). As the disease progress to the chronic form, there will be a decrease in the CD4+/CD8+ ratio (40) and when fibrosis is established the numbers of neutrophils increases. Obviously these studies are performed at one particular time and provide only a fleeting glimpse of dynamic responses in the lungs. The lymphocytosis obtained from the BAL fluid may not be specific to the disease as lymphocytosis has also been found in asymptomatic fanciers and the ratio of CD4+/CD8+ is similar. However there may be functional differences where the lymphocyte cells from the symptomatic group showed greater in vitro responses to pigeon antigens and to polyclonal mitogen phytohaemagglutinin (41). The other cells that are present in the BAL from PFL patients include γδT cells (42), plasma cells and eosinophils (43). Mast cells are also present in BAL fluid specimens and the

Arterial blood gas	Type 1 respiratory failure in severe cases
Blood results	IgG antibody to avian antigens Neutrophil leucocytosis, hypergammaglobulinaemia, rheumatoid factor positive
Chest radiograph	Pulmonary infiltrates
CT scan	HRCT can identify features such as ground glass shadowing and air trapping even when the X-ray is normal.
Gallium scan	Uptake reflects alveolar macrophage activation
TcDTPA lung clearance	Indicates increased alveolar permeability and sub-clinical inflammation (75,76)
Lung function	Restriction of lung volumes, impaired gas diffusion, hypoxaemia and airways obstruction
Bronchial lavage (BAL)	Initial neutrophil alveolitis followed by lymphocytic alveolitis.
Lung biopsy	Diffuse interstitial inflammation of mainly lymphocytes multinucleated giant cells in membranous and respiratory bronchioles, granuloma, constrictive bronchiolitis and fibrosis.

Table 8. Investigations for diagnosing pigeon fanciers lung

number of mast cells in lung tissue correlates with disease activity (44). The number of neutrophil increases when fibrosis develops at the later stage of the disease (45).

Lung biopsy is obtained either by transbronchial lung biopsy or open lung biopsy. The histopathological features are distinctive but not pathognomonic and vary depending on the stage of disease at the time of biopsy and the adequacy of the lung biopsy sample. The characteristic findings include diffuse interstitial inflammation, multinucleated giant cells in membranous and respiratory bronchioles, non caseating granulomas, constrictive bronchiolitis and fibrosis in the later stages of the disease (46). The diffuse interstitial inflammation mainly consists of lymphocytes, macrophages, mast cells and plasma cells. If there is exposure to endotoxins, there may be significant neutrophilic inflammation as well as emphysema.

iii) Diagnosis

The variability in the clinical presentation of PFL and its similarity with other diseases can lead to the underdiagnosis of PFL. The most important pointer towards a diagnosis is exposure to the relevant avian antigen which should be obtained from the initial history taking. The relationship between avian exposure and initial onset of symptoms as well as recurrent episodes related to exposure is often evident. Respiratory and systemic symptoms related to avian antigen exposure with a positive antibody response is usually enough to make a diagnosis without obtaining BAL fluid and lung biopsy. While the BAL fluid is characterised by an accumulation of CD8+ cells, this is not unique to this disorder as this can also be found in BAL of patients with AIDS, collagen-related disorders, silicosis and drug induced pneumonitis. Therefore the diagnosis of PFL can remain difficult and may rely on histopathology. Table 9 lists the common diseases that need to be considered in the differential diagnosis.

In a recent review of diagnostic criteria by a committee they identified six significant predictors of EAA/HP: exposure to the known offending antigen, positive precipitating

Other causes of hypersensitivity pneumonitis
Other causes of diffuse lung diseases such as idiopathic pulmonary fibrosis
Acute bronchiolitis or pneumonia
Acute endotoxin exposure
Asthma
Acute dust toxic syndrome
Allergic bronchopulmonary disease
Pulmonary embolism
Aspiration pneumonitis
Bronchiolitis obliterans organizing pneumonia
Mycobacterial, fungal, viral infections
Chronic beryllium disease
Sarcoidosis
Churg Strauss syndrome
Wegener's granulomatosis
Lymphoma/leukaemia
Bronchiectasis

Table 9. Differential diagnosis of PFL

antibodies to the offending antigen, recurrent episodes of symptoms, inspiratory crackles on physical examination, symptoms occurring 4-8 hours after exposure and weight loss. Exposure to the offending antigen was the strongest predictor for EAA and the probability of having EAA ranged from 0% with none of these features to 98% with all six (47,48).

iv) Management and Treatment

Pigeon fanciers have a strong affinity for their birds and sport. Our data has shown that on average they spend on average 18 hours per week in their lofts looking after 85 pigeons on average. The time spent in the pigeon loft is considerably more during the racing season. They have made a considerable financial and emotional commitment and its social interactions often dominate their lives. It is unlikely that a pigeon fancier would consider giving up their lifestyle and commitment to their sport without looking at other options. They may be reluctant to seek medical attention in fear of being told to give up their birds. Medical staff should understand that giving up the pigeons is not an initial option but only as a last resort if other methods have been found to be ineffective in controlling their symptoms.

The most important intervention in managing this condition is to reduce the amount of avian antigen exposed and inhaled. This can be done in several ways.

1. Reducing the time spent with their pigeons.
   This may be difficult to adhere because fanciers develop their skills and experience as a result of spending time in contact with their pigeons, training and breeding them to develop their homing instinct. The time in contact with the pigeons should be reduced and excessive handling of them discouraged. Someone else can perform the daily cleaning of the loft or taking the pigeons away for training flights.

2. Wearing a suitable mask.

   This will filter out 90-95% of respirable particles therefore reducing the amount of antigen inhaled. In the majority this intervention will dampen the immune response.

3. Wearing a dedicated hat and coat while in the loft.

   Antigenic dusts have also been measured in the home environment (17). This may be carried into the home from the loft on clothing and hair of the fanciers. By wearing a dedicated hat and coat in the loft area this will help to reduce the spread of bloom from the loft area. Fanciers should also wash their hands after handling their birds. These antigenic dusts can persists for up to 18 months in the home environment after removal of birds and may be the explanation where there is a persisted positive avian antibody level despite giving up their pigeons (49-51).

4. Loft management strategies

   Pigeon lofts are generally dusty and fanciers are exposed to significant amount of antigenic dusts which are mainly derived from pigeons and consist of pigeon droppings, feather dust (bloom) and dust from grain feed.

   Lofts using the deep litter method where droppings accumulate, dry out and become fine chalk like powder which absorbs fresh dropping have significantly higher levels of antigenic dust compared to lofts that are cleaned daily which is the more common method (16). Lofts should also be designed for minimising the amount of antigenic dusts being inhaled by fanciers and various loft ventilation strategies can be adopted for this purpose.

There are no randomised double blind placebo controlled trials of corticosteroid therapy in Pigeon Fanciers Lung but a randomised controlled trial in acute farmers lung found that prednisolone improved lung function compared to the control group but there were no difference in the long-term outcome between the two groups (52).

Patients with symptoms should be considered for a trial of steroid therapy to suppress the active immune response. Controlled trials are lacking but there are many anecdotal reports of the beneficial effects of systemic steroids in the acute stage of the disease. Severe attacks may necessitate treatment with oral prednisolone 40-60mg daily with supplement oxygen therapy and other appropriate supportive measures. The effects of steroids on the long term course of PFL are unknown. In one study of PFL, there were no significant clinical outcome differences between cases who were treated with steroids and those who were not (53). Patients with complaints of cough, wheeze and chest tightness with airflow obstruction may benefit from inhaled steroids and beta agonists therapy (54). There is no data from controlled trials regarding the efficacy of the inhaled steroids in PFL. The effectiveness of the steroid trial and antigen avoidance measures can be guided by clinical symptoms, chest radiograph, lung function and circulating antibody levels.

There is evidence that the long term prognosis of farmer's lung is poor (55) where some patient's progression continued despite avoidance of antigen exposure. For example, 24 of 61 farmers with acute HP who stopped farming for 3-5 years noted continued decline in diffusion capacity and total lung capacity (56). There seems to be several predictors of long term decline in farmers such as recurrent episodes, swine confinement areas, exposure to bacterial endotoxin, allergy to mites and fungal infections (57). Progressive deterioration of lung function indicates progressive disease and the changes are likely to be irreversible leading to respiratory failure, cor pulmonale and ultimately death (56-58). Lung transplantation may be the last resort in patients with progressive disease and unresponsive to medical therapy.

There had been reports that therapeutic doses of erythromycin, which has anti-inflammatory properties significantly suppresses the neutrophil influx into the lung, intradermal Arthus reaction, and the expression of intercellular adhesion molecule 1 in experimental HP lesions (59). Therefore erythromycin may be effective in the acute form of HP but this requires further studies.

In children the most common reported cases of HP are due to avian exposure, mainly to domestic birds. The outcome of these children who receive proper diagnosis and treatment is excellent. In the 67 pediatric cases of HP with outcomes, 65 children improved or became asymptomatic, 1 patient was worse, and 1 patient died 9(60).

3. LUNG FUNCTION

During the acute symptomatic phase there is a restrictive ventilatory defect with reduction in gas exchange and this return to normal between episodes. There may be an increase in alveolar-arterial oxygen gradient on arterial blood analysis and oxygen desaturation with exercise suggests early gas exchange abnormalities. This suggests the inflammatory disease is mostly at alveoli level (61-64). However, pathological studies and animal models of EAA suggest that inflammation is not confined to the alveoli but also involves the bronchi and smaller airways (9,65). Subjects with PFL in addition to having the well recognised restrictive defect also had a high prevalence of chronic bronchitis, large airways involvement and peripheral airway obstruction. Whether this is due as part of the immunological response in PFL or as a result of inhaling endotoxins present in the pigeon loft is uncertain. The lung function usually improves in fanciers who cease exposure but the abnormalities in lung volumes and diffusing capacity may persist in some (66)

In chronic disease there is often evidence or persisting restrictive defect, with reduction in gas transfer. When the abnormalities persist or deteriorate despite avoiding antigen exposure, this indicates permanent damage to lung structure and function. If antigen exposure continues this may progress to pulmonary fibrosis, with worsening restrictive defect and gas transfer and ultimately respiratory failure.

4. ANTIGEN

There is a range of antigens that can cause alveolitis but the clinical presentation and subsequent immunological response is similar to all the different antigens. Antigens involved in PFL need to be ideal in size and shape which, when inhaled will penetrate the distal alveoli to initiate an immune and inflammatory response. This is in contrast with asthma where the antigens involved are larger and are deposited proximally in the bronchi causing an IgE reaction in atopic individual. There are many sources of antigen which have been described from pigeon droppings (67), which have been described as the "complete" source of antigens associated with the disease, bloom from pigeon feathers, serum, egg yolk and white, crop fluid and gut wall all of which contain mixtures of protein antigens (68-70).

Pigeon lofts are generally dusty especially during autumn when the pigeon moult and shed their feathers. There is a range of pigeon associated antigens present in the pigeon loft. The main source of antigen is bloom. Bloom is a fine dust produced from feathers by birds that can fly. It appears like talc powder coating the surface of pigeon lofts and covers the hands, hair and clothing of the pigeon fancier. It is an inert keratin protein granule coated with pigeon IgA about 0.75 microns in diameter with the correct aerodynamic size to enter the peripheral airways and alveoli and initiate the immune response. It is at this alveoli level that the disease process is most apparent. Keratin is resistant to enzyme breakdown and may act as an inflammatory foreign body. Fit pigeons trained to race produce bloom in copious amount to keep them dry in wet racing conditions. In contrast birds that do not fly such as duck, chicken and turkey produce minimal amount of bloom explaining the fact that HP among poultry workers is uncommon. Fanciers are exposed to bloom when in the loft when handling them or when the pigeons fly close to them especially during the racing season when they spend more time in the lofts and autumn when pigeons shed their feathers

Droppings and waste materials in the loft are also antigenic source but less potent because they are larger, moist and less readily inhaled. IgA is the major protein antigen in droppings (71) but also contains other less defined antigenic materials. Droppings are also a source of bacterial endotoxin and fungal glucan, which are proinflammatory (21). Disease specific 21-kd proteins from pigeon dropping have been described causing lymphocyte transformation only in subjects with PFL (72). Pigeon intestinal mucin is a major carbohydrate antigen with a high molecular weight glycosylated protein core that is resistant to degradation and likely to be stable in the pigeon loft environment and difficult to clear within the lungs (73,74). Mucin is also found to be abundant in pigeon droppings and bloom.

Pigeon serum is the main source of antigen for use in serological and inhalational challenge studies where the main antigens are gamma globulin proteins. It contains a range of antigens including proteins, glycoproteins and polysaccharides that is detected by the sera of sensitised pigeon fanciers (77,78). It is readily available and contains the major antigens while avoiding problems with contamination of droppings or bloom extracts by microorganisms or other agents (79). There are also other various antigens such as bacterial, fungal, viruses, parasites and mites that have been identified in the pigeon lofts that may cause symptoms mimicking PFL (Table 6).

5. IMMUNOPATHOGENESIS OF PFL

The pathogenesis of PFL involves repeated antigen exposure, immunological sensitisation of the host to the antigen and immune mediated response from the host causing pulmonary and systemic symptoms. The immune response is characterised by interstitial and alveolar inflammation with granuloma formation (80). This appears to involve a combination of immune complex mediated or type 3 reaction and cell mediated or delayed type IV immune response to the inhaled antigens. The delay in the onset of symptoms after antigen exposure and the high levels of specific avian antigen immunoglobulins and complement components in the serum and BAL supports the role

of type 3 reaction or immune complex mediated response. These immune complexes can activate alveolar macrophages and have also been shown to release proinflammatory cytokines, particularly TNFα and IL-1 which causes lung inflammation (81-83). Immune complex induced lung injury can be blocked by an antibody to recombinant TNF and related proinflammatory cytokines in experimental animals confirming that these cytokines are released by immune complexes (18).

There is strong evidence that type 4 allergic reaction or cell mediated immunity are also involved in the pathogenesis of PFL. Animal models support the importance of cell mediated immunity in HP. Cultured lymphocytes passively transferred from sensitised animals to unexposed non sensitised animals results in disease similar to humans when the naive animals are subsequently challenged with inhaled or infused intrapulmonary antigens (84). Interferon gamma is the prototype of Th1 cytokines activating alveolar macrophages and is a key factor in the events that favour the local immune responses in the lung. Studies using 'knockout'mice incapable of expressing the gene coding for interferon gamma, these mice do not develop significant amounts of granuloma formation in the lungs after exposure to antigen (85). Pathologically, granulomatous interstitial pneumonitis with variable degrees of distal bronchiolitis obliterans is the characteristic findings in HP and this inflammation is caused by T cell mediated immune response to antigens entering the lungs. Therefore, the evidence of the importance of T cell mediated immune response includes (a) antigen reactive T cells with an activated phenotype that are present in the blood and lung. Peripheral lymphocytes from symptomatic fanciers exhibited spontaneous proliferation when challenged in vitro with pigeon serum antigen but lymphocytes from asymptomatic fanciers showed no significant proliferation to antigen (b) biopsy features typical of T cell mediated immune response and (c) in animal models, where the possibility of transferring the disease to healthy animals by adoptive transfer of T cells.

The nature of the inflammatory infiltrate reflects the stage of disease. Samplings of airway cells and fluid by bronchoalveolar lavage (BAL) have improved our understanding of the immunopathology of PFL. However these studies need to be

interpreted carefully as the studies varies in defining a characteristic positive test, the time of exposure and sampling are different and PFL are grouped together with other HP. BAL shortly after antigen exposure demonstrates an acute neutrophil infiltrate (34) which corresponds with the clinical phase of acute symptoms. This infiltrate becomes predominantly lymphocytic after 24-48 hours (86,87) and the proportion of lymphocytes recovered by BAL correlates with the degree of lung inflammation (88-91). The 2 mechanisms that account for the lymphocytosis within the alveolar space are the cellular redistribution from peripheral blood to the lungs and in situ proliferation. In acute disease these lymphocytes are predominantly CD4 T-cells but in more chronic disease CD8 T-cells predominate, along with variable numbers of plasma cells, which is not normally found in BAL. Both cellular redistribution from peripheral blood to lung and in situ lymphocyte proliferation contribute to the increase in CD4+ and CD8+ T cells in the lung reflecting the intensity of the alveolitis and the local immunoglobulin levels (91,92). The CD4+/CD8+ ratio is generally lower than that in the healthy person although this is variable depending on several factors such as dose of inhaled antigen, disease stage and other non-specific irritants present in the environment. There is general mobilisation of the immune apparatus of the lung. Bronchus-associated lymphoid tissue (BALT) and B cell expansion is induced and is probably maintained by persistent inflammation. The expression of nuclear proliferation antigen Ki-67 on the centroblasts located in the BALT germinal centres indicates intense local antibody production (93).

B lymphocyte involvement is suggested by the increase amount of antibody response to inhaled antigens resulting high titres of IgG antibody in peripheral blood, BAL fluid, sputum and saliva in the majority of fanciers. Positive IgA and IgM antibodies to avian antigen are also found to be higher in the symptomatic pigeon fanciers (94). The higher antibody response correlates with the severity of the disease. However many antibody positive fanciers remain asymptomatic suggesting antibody appears necessary, but not sufficient to produce disease.

After the antigen is inhaled, it binds to IgG antibody and the immune complex initiate the complement cascade and the resulting C5 activates alveolar macrophages which secretes

chemokines such as MIP-1α and cytokines. MIP-1α promotes the differentiation of CD4+ Th0 cells to Th1 cells and acts as a chemotactic factor for macrophages and lymphocytes. Alveolar macrophages that is important in the pathogenesis of PFL, process and presents antigen to the CD4+ cells in the early phase. A variety of cytokines are produced including pro inflammatory and regulatory cytokines. Pro inflammatory cytokines such as TNF alpha, IL-1 and IL-8 are produced explaining the acute symptom of fever and neutrophilia (95). In comparison with other idiopathic pulmonary fibrosis, macrophages from patients with HP secrete more TNFα and less IL-1. Regulatory cytokines IL-12 and IL-18 are also produced which can polarize lymphocytes towards a TH1 type bias response. Regulatory cytokines such as IL-10 are known to counteract many of the biological effects of interferon gamma suggesting that IL-10 plays a role in damping down the inflammatory process (96) and granuloma formation. Knockout mice and adenovirus-mediated gene transfer of IL-10 to the liver of these mice showed that IL-10 has important anti inflammatory properties and the lack of this cytokine leads to increase granulomatous inflammation response (97). The distribution of these pro inflammatory and regulatory cytokines and the genetic polymorphism (14) that determines the amount produced at each stage of stimulation may determine the clinical outcome. The crucial distinction between CD4+ and CD8+ T cells pertains to recognition of antigens presented by different major histocompatibility complex molecules. Activated macrophages also have increase expression of adhesion molecules (CD80, CD86) to allow inflammatory cells into the lung tissue and ICAM-1 that is necessary for lymphocyte recruitment in the lungs (98,99). The soluble ICAM-1 levels are also increased in the BAL fluid (100) and this correlated with physiological indices of lung inflammation all of which normalise after cessation of antigen exposure (101).

More advanced disease is characterised by increasing fibrosis with thickening of the alveolar septa and basement membrane (102). Activated alveolar macrophages express increased amount of TGF-β, a potent stimulator of fibrosis and angiogenesis (103). Expression of the Fas ligand and the CD40 ligand systems are also involved in the development of fibrosis (104). This fibrosis appears to be related to the proportion of activated neutrophils (45) and increased numbers of mast cells (105,106).

6. AIMS OF THESIS

Most of the studies performed are done on patients presenting to clinics or on volunteers attending a conference. A part of this research is to look at a cohort of pigeon fanciers in a particular area and determine weather there are any changes in lung function, symptoms and immunological status in a 5 year period. The relationship between lung function and immune responses among these pigeon fanciers were also explored.

The trend of immune sensitisation among a large group of pigeon fanciers were also studied to determine whether there has been any substantial change in the immune status over a 13 year period and how these changes occurred.

The presence of serum antibody to avian antigen does not necessary imply presence of disease in the individual as many antibody positive fanciers remain asymptomatic. This study looked at the evidence of subclinical disease where underlying inflammatory response is ongoing despite no clinical symptoms experienced. The type of immune response, clinical presentation and subsequent progression of disease are likely to be influenced by various environmental and constitutional factors. These factors determine the susceptibility of these fanciers in developing PFL. This thesis attempts to identify the role of these environmental and constitutional factors in determining the clinical course of disease and the ability to mount an immune response. The association of serum IgG antibody with clinical symptoms, incidence of disease and lung function were also identified.

Role of environmental factors studied

- Antigen exposure which can be measured – number of birds kept, average weekly exposure and number of years involved
- Cigarette Smoking

Role of constitutional factors studied

- Age
- Avian antibody production
- Proinflammatory and regulatory cytokines and role of CD8+ T lymphocyte

CHAPTER 2

5 -YEAR LONGITUDINAL STUDY IN A COHORT OF PIGEON FANCIERS

1. SUMMARY

The immuno-pathogenesis of PFL is unresolved. Most studies rely on subjects presenting at clinics and proper epidemiological evaluation is lacking. Bourke et al study evaluated 24 patients diagnosed with the acute form of pigeon fanciers lungs 10 years after the original diagnosis and found that the majority had stable lung function and improved symptoms despite continued avian antigen exposure (2). However, there are no epidemiological data on changes in lung function and immunology in a population of active pigeon fanciers over a period of time. In this study, a cohort of 41 pigeon fanciers had serial lung function, serum antibody IgG and associated symptoms related to inhaled avian antigens measured over 5 years.

This study found that between 1997 and 2002 there was a significant reduction in FEV1 ($T = -3.8$, $p = 0.004$) and FEV1/FVC% ($T = -3.22$, $p = 0.003$). 22 subjects were seropositive in 1997 and a further 3 subjects showed evidence of new sensitisation in 2002 and there was no significant increase in paired mean IgG antibody titre. There was no significant change in the symptoms reported. The FEV1 % correlated inversely with peripheral blood CD8 lymphocyte proportion ($r = -0.412$, $p < 0.01$) and the IgG antibody titre also correlated inversely with the CD4/8 ratio ($r = -0.4$, $p = 0.02$). 11 subjects had symptoms suggestive of PFL in 2002 compared to 13 subjects in 1997.

Serial lung function in statistically determined cohort of these 41 pigeon fanciers seems to deteriorate significantly despite no change in symptoms reported. These changes are associated with underlying immune dysfunction involving the imbalance of T-helper and T-cytotoxic lymphocytes. This study also demonstrated that sub-clinical inflammatory changes are common among pigeon fanciers and may be predictive of disease progression.

2. Introduction

There is little data on the epidemiology and disease prevalence of pigeon fanciers lungs. There are studies on fanciers with positive antibody response to avian antigens and disease where the prevalence varies between 11-21% (110-113). These figures are wide ranging as the population selection, method of measuring antibody levels and criteria for alveolitis differs from each study. The clinical presentation of PFL also varies and may be confused with other diagnosis. These studies also may have inadvertently excluded symptomatic subjects who are unwilling to be involved in fear of being told to give up their pigeons. Despite this, even a conservative estimate of prevalence would generate a large number of cases since the number of registered pigeon fanciers in the UK alone is 80,000 (114). In comparison with Farmer's lung the incidence reported in the farming communities ranges from less than 1% to 6% in farmers (115,116).

However most fanciers remain asymptomatic despite being antibody positive for avian antigen. Most studies are performed on volunteers or patients attending clinics. Many symptomatic fanciers are afraid to be told to give up their pigeons and avoid doctors, therefore a study on the longitudinal course of this disease is difficult to complete (117). The purpose of this study is to assess the longitudinal course of a cohort of active pigeon fanciers over a 5 year period.

The classical abnormalities of pulmonary function tests that have been described are associated with a restrictive defect with loss of lung volume, hypoxaemia and abnormality of gas exchange with a low diffusion capacity and reduce KCO suggesting mostly alveolar involvement (118). However, other studies and animal models of EAA suggest that inflammation is not confined to the alveoli but also involves bronchi and smaller airways (119). It has been shown that both in pigeon fanciers and farmers there is a high prevalence of chronic bronchitis (9,10). This increases as antibody levels rise after the typical risk factors such as smoking and dusty atmosphere have been excluded.

This study looked at the large and peripheral airways involvement over a 5-year period measured by spirometry. The immune responses were measured which included IgG avian antibody level and T lymphocytes subsets in peripheral blood in 1997 and its correlation with lung function. The symptoms experienced by the fanciers related to their hobby were also recorded

3. Methods

110 Pigeon fanciers from the North West Federation Fanciers were randomly selected from the club's register of 330. 81 fanciers agreed to participate in the study in 1997. Letters were sent out to the 81 fanciers inviting them to participate in the repeat study in 2002. 41 out of the 81 fanciers agreed to be involved in the repeat the study in 2002. Data was collected at pigeon marking sessions where fanciers brought their pigeons to be marked before racing day. Each subject filled in a self-completed questionnaire, performed a spirometry test and provided a 5ml blood sample.

The self-completed questionnaire (Appendix A) was done under medical supervision to ensure accuracy of data. The questionnaire included clinical information relating to amount of exposure to pigeons, self regulatory measures, symptoms related to pigeon exposure, smoking history and other respiratory symptoms in accordance with the MRC questionnaire on chronic bronchitis. Spirometry was performed with a Vitalograph Alpha machine and based on the ATS guidelines on performing spirometry testing (Table 10) (120).

IgG antibody to avian antigen were measured by enzyme-linked immunosorbent assay (ELISA). The standard protocol is shown in Appendix B.

Subjects are regarded as having pigeon fanciers lung if they have at least one classical respiratory (dyspnoea, cough, wheeze or chest tightness) and one systemic symptom (fever, sweating or flu like symptoms) occurring on a regular basis following contact with their pigeons with serological evidence of avian sensitisation. Statistical analyses were

performed using Minitab software. The paired T test was used to evaluate the differences in lung function in 1997 and 2002. Pearson correlation coefficient was used to determine the correlation between lung function measured and immune responses.

4. Results

Of the original 81 fanciers who took part in 1997, 28 (34%) were smokers. Table 11 shows the baseline characteristics of pigeon fanciers in 1997 and 2002. Table 12 and 13 shows the distribution of smokers and non-smokers in 1997 and 2002. In both groups, smokers had a significantly lower IgG antibody level and a lower mean age. There were also more subjects with no evidence of sensitisation to avian antigen in the smoking group.

Out of the 41 fanciers in 2002, 22 fanciers were positive for antibody response to avian antigen in 1997 and a further 3 subjects showed evidence of new sensitisation in 2002. Among the smokers (10 fanciers) in 2002 only 2 was positive for IgG antibody response (in both years) but both had high levels of 58ug/ml and 79ug/ml with significant symptoms of PFL. Interestingly one subject who smoked and had no antibody response in 1997, stopped smoking and his IgG level was 15ug/ml in 2002 with minor respiratory symptoms.

5 fanciers stopped keeping pigeons by 2002. Two of the fanciers had no IgG antibody response during both years. The other three had significantly elevated levels of IgG and symptoms in 1997 and the levels dropped and they became asymptomatic in 2002 (subject 1,14 and 25)

Subject	IgG 1997 active	IgG 2002 Non active
1	100	50.7
14	70	7
25	55	18.2

The mean FEV1, FEV1/FVC% and FEF25-75 for the 41 fanciers involved in both 1997 and 2002 study are illustrated in table 14.

Between 1997 and 2002 there was a significant reduction in FEV1 (T = -3.8, p = <0.05), FEV1/FVC% (T = -3.22, p = 0.003) and FEF25-75 (T= 3.03, p= 0.003) in the study group. However there was no significant increase in paired mean IgG antibody titre. Although there was a significant decrease in FEV1, FEV1/FVC ratio and FEF25-75 during the 5 year period, this did not translate into any noticeable deterioration in symptoms experienced among the fanciers. The lung function measured by spirometry also did not correlate with serum IgG antibody.

FEV1% measured	1997	r = -0.29	p = 0.08,	2002	r = -0.21	p = 0.18
FEV1/FVC ratio	1997	r = -0.04	p = 0.82,	2002	r= -0.21	p = 0.21
FEF25-75	1997	r=-0.028	p = 0.86,	2002	r= -0.29	p = 0.06

In 1997 FEV1% correlated inversely with peripheral blood CD8 lymphocyte proportion (Figure 1, r= -0.412, p=0.010). The IgG antibody titre correlated with CD 8 lymphocyte count (Figure 2, r=0.34, p=0.01) and was inversely correlated with CD4/CD8 lymphocyte ratio (Figure 3, r=-0.40, p=0.02).

13 subjects in 1997 had symptoms of acute intermittent non progressive PFL. 2 of the subjects stopped keeping pigeons (subject 1 and 14) and no new subjects described symptoms of PFL. There was no significant difference in antigen exposure measured by number of birds kept and number of weekly hours spent in pigeon lofts within the 2 groups (Table 15).

1. Spirometry calibrated on site
2. Test explained to subject
3. Prepare subject – ask about recent illness, medication etc
4. Instruct and demonstrate test to subject – correct posture and manoeuvre technique
5. Subject performs a minimum of three acceptable FVC manoeuvre meeting the acceptability and reproducibility criteria.
6. The highest reading at any manoeuvre are stored for analysis

Table 10. ATS guidelines on performing spirometry testing (120)

	1997	**2002**
Age	53.5	58.5
No of birds	57	65
Weekly hours in loft	21	23
FEV1	3.28	2.91*
FEVC1/FVC%	76.3	72.9*
FEF 25-75	2.94	2.54*
IgG (ug/ml)	27.2	31.2
Number of fanciers sensitized	22	25
Symptom score	2.3	2.1

Table 11. Characteristic of pigeon fanciers in 1997 and 2002 (values are means) *$p<0.05$

	n	Mean age	IgG (ug/ml)	Seronegative
Smokers	28	48	7.4	16 (57%)
Non smokers	53	54	39.2	14 (26%)

Table 12. Distribution of smokers and non smokers with mean age and IgG levels in 1997

	n	Mean age	IgG (ug/ml)	Seronegative
Smokers	10	56	14.1	8 (80%)
Non smokers	31	60	34	8 (25%)

Table 13. Distribution of smokers and non smokers with mean age and IgG levels in 2002

	FEV1 (litre)	FEV1/FVC%	FEF25-75 (litre)	Mean IgG (ug/ml)
1997	3.28	76.3	2.94	27.2
2002	2.91	72.9	2.54	31.2

Table14. Lung function and IgG antibody results in 41 pigeon fanciers (values are mean)

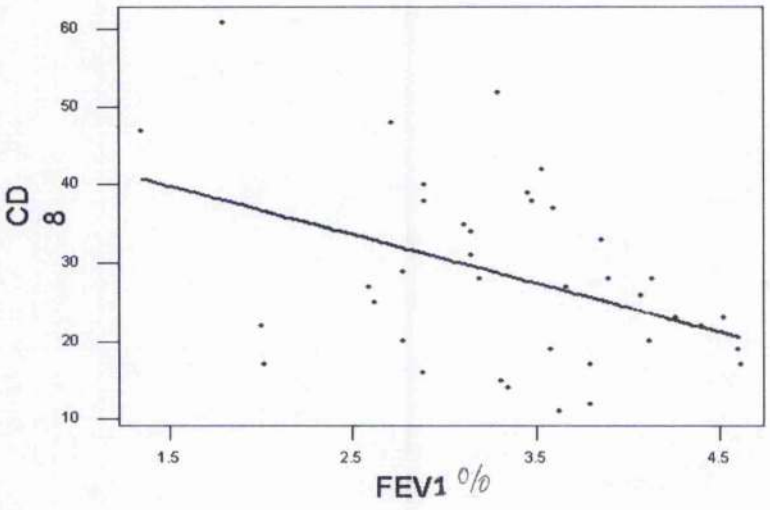

Figure 1. Inverse correlation between FEV1% and CD 8 lymphocyte count

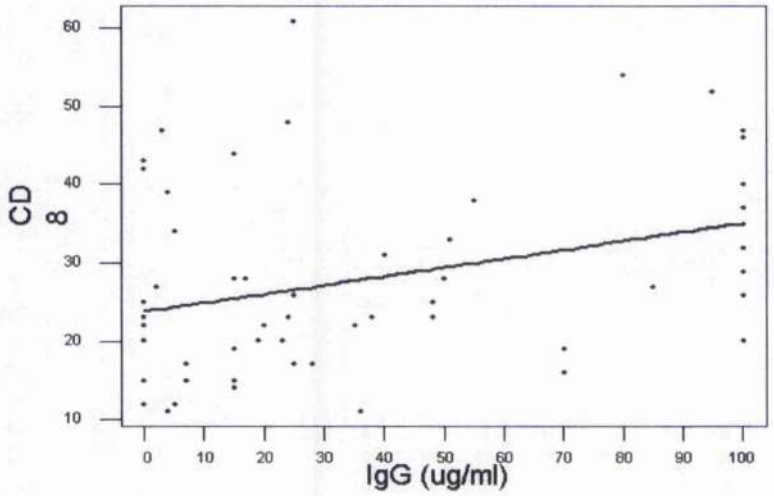

Figure 2 IgG antibody correlates with CD 8 lymphocyte count

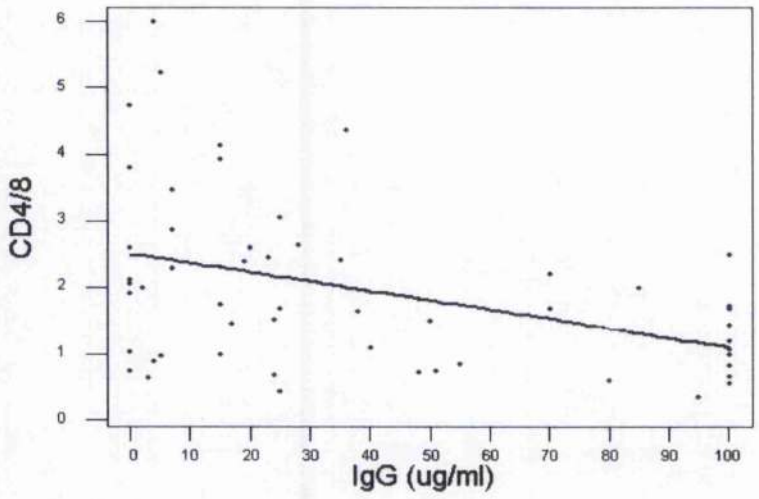

Figure 3. Correlation between CD4/CD8 and IgG antibody

	Average no of birds	Average weekly hours in loft	% Wearing mask
1997	57	21	38
2002	65	23	36

Table 15 Antigen exposure and % of fanciers wearing mask

5. Discussion

Serial lung function measured by spirometry in a cohort of pigeon fanciers seems to deteriorate significantly over a period of 5 years. However despite these statistically significant changes, the fanciers do not complain of any noticeable deterioration in their symptoms. This may be due to the initial normal lung function among pigeon fanciers and they are able to tolerate the accelerated decline in FEV1 and reduction in FEV1/FVC ratio and FEF25-75 without developing any appreciable respiratory disability. This is further complicated by self-regulatory measures when they develop symptoms where fanciers may try to reduce antigen exposure by wearing mask and other attire (coat, hat and gloves) as a compromise between their health and hobby. Out of the 41 fanciers in 2002, 15 wore mask in the loft and all of them apart from one fancier found their mask useful in controlling their symptom. Despite having intermittent non-progressive symptoms the majority of fanciers continue with their commitment to their pigeons. Certainly there are many reports of fanciers with symptoms who continue to be exposed to avian antigen exposure and their symptoms regresses rather than progress despite high antibody levels. Animal studies have also shown a decrease in pulmonary inflammatory response where repeated antigen challenge is given and the antigen is metabolised differently in sensitised animals (121).

Smokers have a significantly lower IgG antibody levels and more subjects without antibody response. Smoking appears to suppress the pulmonary immune response and the reported incidence of PFL among smokers was found to be lower in previous studies. The association between smoking and antibody response will be explored further in chapter 4.

These lung function changes are associated with underlying immune dysfunction involving the imbalance of T-helper and T-cytotoxic lymphocytes. The FEV1 measured in 1997 correlated inversely with CD8+ lymphocytes. Although there was no correlation between the deterioration in lung function and IgG antibody responses, the changes in antibody response were associated with CD 8+ lymphocyte count and the imbalance of T lymphocytes subsets. Recently there has been interest in T lymphocytes subset

abnormalities in peripheral blood, which might be involved in the pathogenesis of airflow limitation (122). Studies have shown a relative decrease in CD4+ T lymphocytes and increase of CD8+ T lymphocytes in smokers and non smoking subjects with COPD.
The T lymphocytic infiltrate in lung biopsies of patients with PFL also shows a reduce ratio of CD4:CD8 suggesting the imbalance of the T lymphocyte population may have a role in the pathogenesis of PFL (123-125). The role of the CD8+ T lymphocytes may be important as it can produce cytokines such as gamma interferon, which may have a role in the tissue remodelling associated with lung function abnormality in determining progression of disease.

There may be several reasons why the antibody response did not completely subside especially in subject 1 and 25. These subjects may well still be exposed to pigeons while socialising with other fanciers and they would certainly have had some avian exposure while attending the pigeon marking sessions. There may also be other additional exposure to avian exposure from bird feathers in duvets, pillows and soft furnishings. Antigenic dusts have also been measured within the home of fanciers and can persists for up to 18 months in the home environment after removal of pigeons and this may explain the slow resolution of IgG avian antibody response or clinical improvement (126)

This population studied showed that the prevalence of probable PFL in that community was around 25% and sub-clinical inflammatory changes are common among the pigeon fanciers and this may be predictive of disease progression

CHAPTER 3

TREND OF IMMUNE SENSITISATION AMONG PIGEON FANCIERS

1. SUMMARY

There are 80,000 registered pigeon fanciers in the UK. Many of them have been involved in this hobby for a long time. The Blackpool Pigeon Show is an annual event where pigeon fanciers from all over the country gather to participate in this event. The British Pigeon Fanciers Medical Research Team have been part of this annual event where fanciers are able to seek advice and have their blood tested for evidence of sensitisation towards avian antigen. This study looked at the trend of sensitisation among the pigeon fanciers attending the Blackpool Show over a 13 year period.

There was an increase in the number of fanciers volunteering to provide blood samples over that period and a total of 4045 blood samples were collected. In 1991 the majority of fanciers had no evidence of sensitisation and less than 30% had moderate or high levels of sensitisation. This is in contrast to 2003 where over 70% had moderate or high levels of sensitisation. Therefore there seems to be a change where the majority of pigeon fanciers now show a positive antibody response towards avian antigen. This response correlates with the symptoms experienced with their pigeons where the strongest responses are the most symptomatic. The majority of fanciers perform some form of self regulatory measures to reduce avian antigen exposure and are able to continue with their hobby despite evidence of sensitisation. When the data from Blackpool were compared to different centres and populations, the level of sensitisation among the fanciers seems to be similar.

2. Introduction

No studies have been performed to look at the changes in the trend of immune sensitisation among pigeon fanciers over a period of time. It has been known for a long time among pigeon fanciers about the intolerance of their birds that was different from orthinosis (127,128). This intolerance has been described in 1897 among the gaveurs de pigeons who force fed thousands of pigeons for market in Paris. They described the typical pattern of pigeon fanciers lung (129). However, Reed and colleagues described the first clinical report describing alveolitis caused by avian exposure in 1965 (130). By the 1980's there were still many fanciers doubting the existence of PFL and potential damage to their health as they contribute their symptoms to being part and parcel of their hobby mainly because they are afraid to be told to give up their pigeons. The British Pigeon Fanciers Medical Research Team has been instrumental in educating pigeon fanciers of the existence of the disease and potential detrimental to their health if symptoms are ignored. With the development of facemasks many fanciers are able to reduce their exposure to avian antigen and experience less symptoms whilst continuing with their hobby.

Apart from providing education, The British Pigeon Fanciers Medical Research Team also has been offering fanciers a chance to check their antibody levels to avian antigen. In this study we reviewed the trend of IgG antibody response among pigeon fanciers from the data collected from pigeon fanciers attending various pigeon shows and samples sent by their GP were reviewed. The Blackpool Pigeon Show is the biggest pigeon show in the UK and most of the data were derived from Blackpool. This data were compared with the data from Birmingham and Newcastle in 2000. Data from samples received from GPs were also reviewed.

3. Methods

Fanciers attending pigeon shows were invited to voluntarily fill a self-completed questionnaire (Appendix A) and provided a 5ml blood sample.

Over 4045 samples from Blackpool Pigeon Show in January each year were collected from 1991-2003 (Figure 4). The samples from Blackpool in 2000 were compared with samples collected in Newcastle and Birmingham in 1999 and the samples received from GPs. From this data the trend of immune sensitisation and symptoms over this period were analysed.

4. Results

The number of volunteer pigeon fanciers involved increased over the 13-year period.

There was a significant increase in pigeon fanciers with high levels of sensitisation over the 13-year period (Figure 5). In 1991, 48% have little or no evidence of avian sensitisation and only 28% have moderate or high levels. In contrast 73% of fanciers have moderate or high sensitisation and only 20% have little or no response in 2003 (Figure 6).

In a cross section review (n=612) of three different areas and population interestingly the level of sensitisation among pigeon fanciers is similar (Figure 7). When compared to the samples received from GPs all over the country from their patients attending the clinics the degree of immune response seems similar (Figure 8).

There was an overlap of IgG antibody responses between symptomatic and asymptomatic fanciers. However, the higher IgG responses, the more likelihood of fanciers experiencing symptoms and the strongest response occurs in the most symptomatic individuals. When the IgG responses were correlated with the symptoms complained by fanciers, a pattern of symptoms recognition emerges as in table 16.

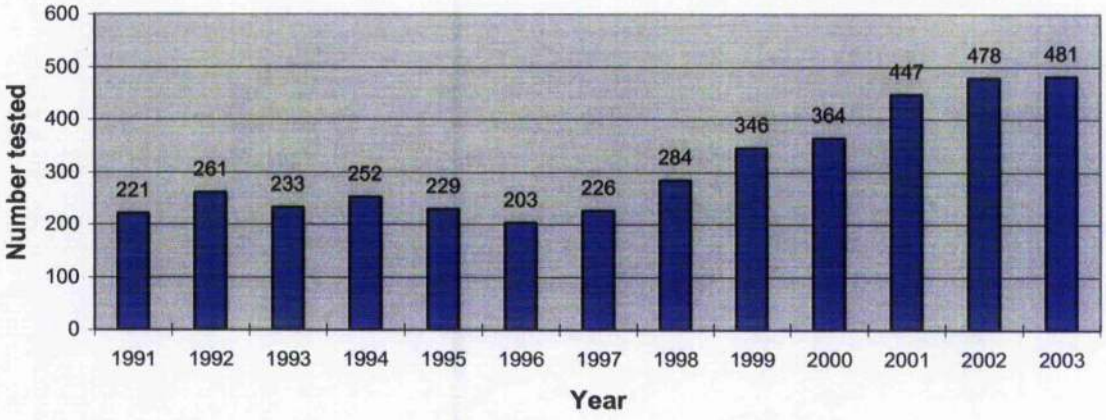

Figure 4. Number of fanciers tested each year between 1991-2003

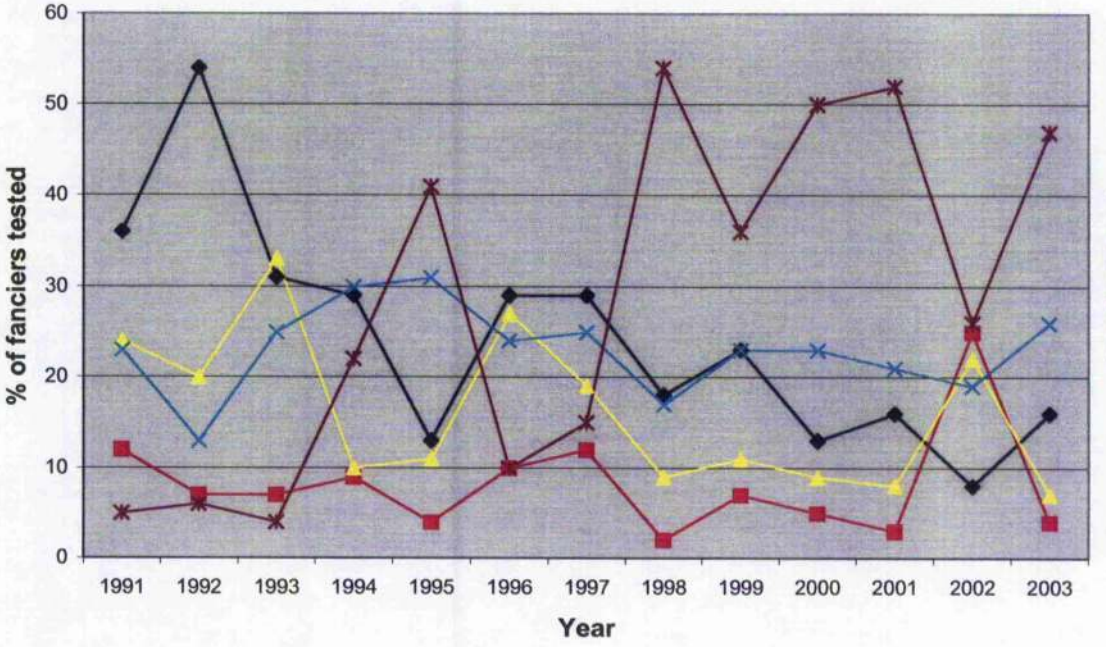

Figure 5. Trend of immune sensitisation between 1991-2003

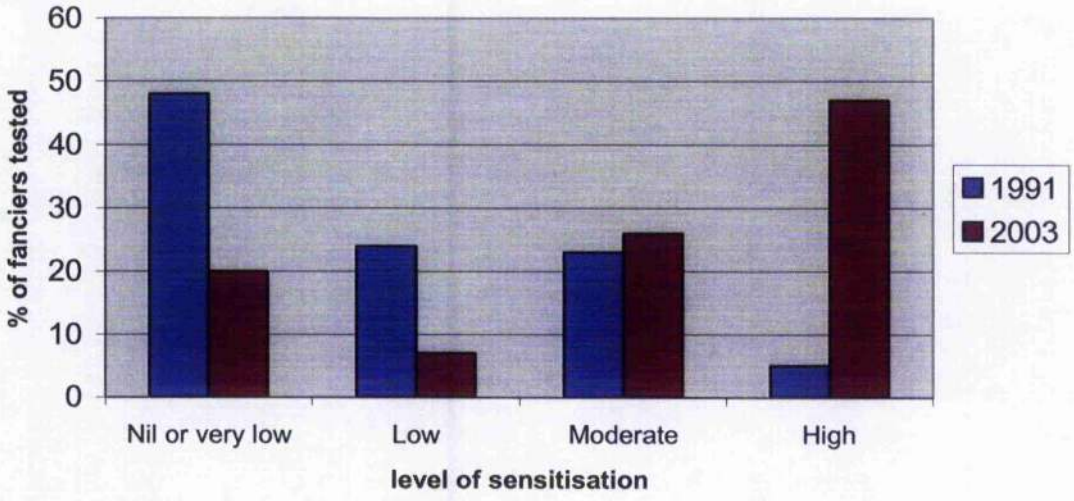

Figure 6. Comparison of level of antibody response between 1991 and 2003

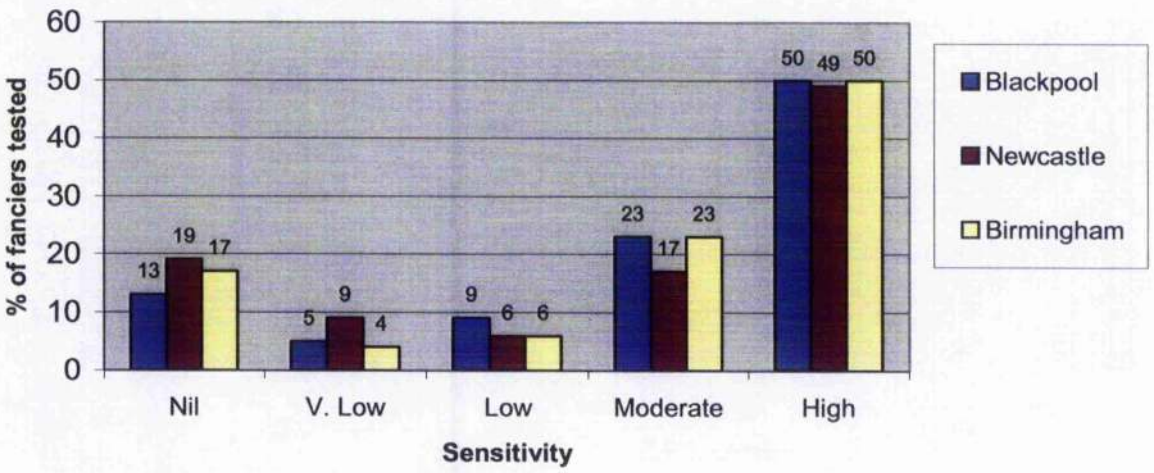

Figure 7. Blackpool 2000 compared with Birmingham 1999 and Newcastle 1999

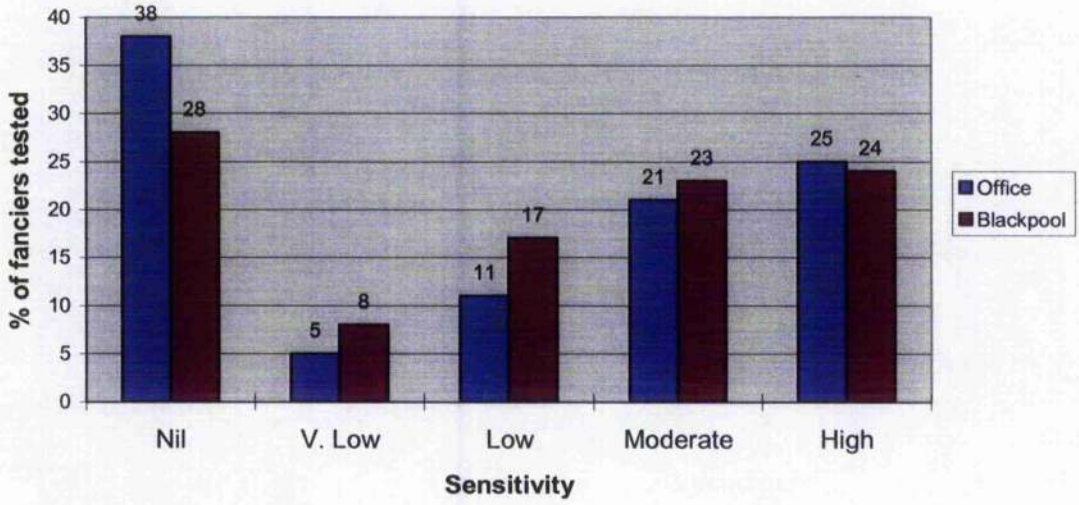

Figure 8. Comparison between samples sent to office 1985-2000 & Blackpool samples 1991-2000

Antibody level (ug/ml)	Degree of sensitisation	Symptoms reported
0-5	Not significant	None
6-20	Mild	Usually none recognised
21-50	Moderate	Intermittent
Above 50	High	Most aware of symptoms
Above 100	Very High	Almost always present

Table 16. Significance of IgG antibody levels and correlation with symptoms

5. Discussion

PFL often goes unrecognised and frequently misdiagnosed as a chest infection, airway disease or idiopathic lung disease as the mode of presentation can be similar to these diseases. The majority of fanciers are concerned with their health and are aware of the association of exposure to their birds and possible symptoms that may develop.
The increase in the numbers of volunteers involved over the 13-year period reflects the increase awareness in the disease and their efforts to monitor their antibody response when they perform self-regulatory measures to control avian antigen exposure as they were informed of their antibody results and appropriate medical advice were also given with the results. There seems to be an increase in the trend of immune sensitisation among pigeon fanciers over the 13-year period. On average 10-15% of fanciers attending each year had already had their IgG levels checked in the past. Whether this reflects a true increase in avian sensitisation in this community or an increase in awareness of the disease and their efforts to monitor their antibody response with measures taken to reduce antigen exposure requires further research. The similar antibody response obtained from 3 different areas and populations (Blackpool, Newcastle and Birmingham) and with the congruous results from patients attending their GP may well suggest the prevalence of sensitisation among the pigeon fancier community.

This study has demonstrated that the majority of active pigeon fanciers do produce an antibody response. Symptomatic fanciers also tend to produce the strongest response. The vast majority of fanciers would experience the acute non progressive disease and continue with their hobby with some adjustment to reduce antigen exposure. Fanciers with acute progressive disease would have little choice but to give up their hobby because of the severity of symptoms. Those with little noticeable symptoms despite an antibody response are at risk developing insidious form of the disease and should be regarded as having subclinical disease and should be encouraged to take steps to reduce antigen exposure. Further prospective studies measuring longitudinal antibody measurement may answer the question of whether these fanciers are at risk of developing clinical disease.

CHAPTER 4

FACTORS AFFECTING ANTIBODY RESPONSE

1. SUMMARY

The majority of pigeon fanciers exposed to avian antigens demonstrate antibody and lymphocyte response. Fanciers that produce the strongest response tend to develop clinical symptoms requiring some adjustment to their daily activities with their pigeons. However not all fanciers develop clinical symptoms despite evidence of sensitisation. Therefore antibody and lymphocytes seems necessary but not sufficient to cause clinical disease. Other factors are likely to be involved in the development of disease. Various constitutional and environmental factors have been identified that may influence the outcome of an individual of developing disease or not (Table 7).

Specific IgG antibody to avian antigen is an important component in the diagnosis of PFL. It is a good marker for monitoring avian exposure and proves recent exposure to avian antigen. In this study various constitutional and environmental factors are measured against the immunological response in pigeon fanciers measured by avian IgG antibody. Smoking is one of the strongest factors that suppress the antibody response although it appears to be reversible. The ability to mount an antibody response increases with age but is not influenced by the amount of avian exposure measured by number of birds kept, years of involvement in the hobby and the amount of hours spent in the loft in a week. This suggests that other factors are more important than the amount of antigen exposure itself in determining the outcome of the immunological response.

The incidence of PFL also increases with a higher antibody response and the number of pulmonary and systemic symptoms experienced by the fanciers correlates with the production of antibody. However there was no relationship established between IgG antibody and lung function in the fanciers studied.

2. Serum IgG antibody response to avian antigen

Pepys et al (1961) first described the association between antibody and extrinsic allergic alveolitis in a farmer with precipitants of an extract of thermophilic actinomycetes grown from mouldy hay. Reed at al (130) described the significance of serum precipitants against pigeon serum and the association of pigeon exposure and interstitial lung disease. IgG remains the predominant antibody produced although IgM and IgA classes are also produced in pigeon fanciers with or without evidence of symptoms of PFL. These antibodies can also be detected in bronchoalveolar lavage, sputum and saliva in individuals keeping pigeons (131)

Serum precipitating antibody was considered a valuable diagnostic index for hypersensitivity pneumonitis (HP) in a recent review of the diagnostic criteria for HP. The diagnostic significance of these precipitants has been questioned in the past as a high incidence of serum antibody has been reported in asymptomatic individuals exposed to the relevant antigen (132-136). Due to its simplicity, the identification of precipitating antibodies by countercurrent immunoelectrophoresis remains the most widely used technique to aid the diagnosis of HP. However this technique provides no quantitative information on antibody concentrations or immunoglobulin classes and lacks analytical sensitivity. Cases of undiagnosed HP have been reported attributed to wrong choice of antigens, underconcentrated sera or improper quality control (137), as there are no standard procedures for this assay. In one study it showed that false negative are common where 30-40% of patients with proven farmers lung disease had no detectable precipitants to commonly tested antigen including Saccharopolyspora rectivirgula, Aspergillus species and Thermoactinomyces vulgaris (138).

It has been recommended recently that the assessment of interstitial lung disease should include testing for antibody against antigen associated with HP and these serological tests should be quantitative to improve national quality control. (135). Accordingly, enzymeimmunoassays methods that are more sensitive, reliable and quantitative are replacing the precipitant technique (134). Studies have confirmed by using this

quantitative method there is a difference in the mean IgG antibody levels between fanciers with PFL and asymptomatic fanciers (139-140). However there is still an overlap of values where some asymptomatic fanciers have elevated IgG levels and a small number of fanciers showing undetectable antibody levels despite describing classical symptoms of PFL.

Using this sensitive method, in this section the levels of IgG antibody levels against pigeon gamma globulin are measured in a large population of pigeon fanciers. This is to assess the humoral response to avian antigens and the influence of a variety of other factors in affecting this response.

Factors measured against antibody response in pigeon fanciers

1. Smoking
2. Age
3. Avian exposure –number of birds, hours spent with bird, years of exposure,

The association of antibody with symptoms and disease

1. Profile of antibody levels
2. Incidence of PFL and association with antibody response
3. Antibody response and lung function

3. Factors measured against antibody response in pigeon fanciers

i) Cigarette smoking

There is a diverse clinical and immunological response among pigeon fanciers exposed to avian antigens associated with PFL. Only a proportion of these pigeon fanciers develop the disease and some continue to remain seronegative for antibody production.
There is a reduced prevalence of PFL and other HP among cigarette smokers. Smoking appears to impair the humoral immune responses as smokers produce lower concentration of serum and BAL fluid IgG to avian antigens compared to non-smokers.

In the survey of 413 pigeon fanciers there were 91 smokers, 210 non-smokers and 113 ex smokers. Table 17 shows the distribution of IgG antibody level against avian antigens and the % of antibody positive subjects for each of the group. The mean antibody level in the smoking group is 32.4 (3-57) ug/ml and is significantly lower compared to non smoking group titre of 54.8 (33-75) ug/ml. The mean antibody level for ex smoker was 54.1 (29-75) ug/ml suggesting that this antibody response is restored in subjects who stopped smoking and this immune impairment due to smoking is reversible. There were also more antibody positive individuals in the non-smokers and ex smokers.

ii) Age

The ability to produce a serum antibody response to avian antigens as a function of age was investigated among the 413 subjects. The mean age was 53.8 years and ranged between 15 and 83 years old. Figure 9 showed the age of the population appeared to be normally distributed. The antibody levels among the each age group are shown in table 18 and figure10. As expected, the number of years involved in the hobby increased with age. There is a significant correlation between age and IgG response (Figure 11, r=0.115, p=0.019).

	Smokers	Non smokers	Ex smokers
Number of fanciers	91 (22%)	210 (51%)	113 (27%)
Antibody positive	64 (70%)	192 (91%)	101 (89%)
Antibody level (ug/ml)	32.4	54.8	54.1

Table17. Number of antibody positive and mean IgG antibody response in the smoking, non smoking and ex smokers group

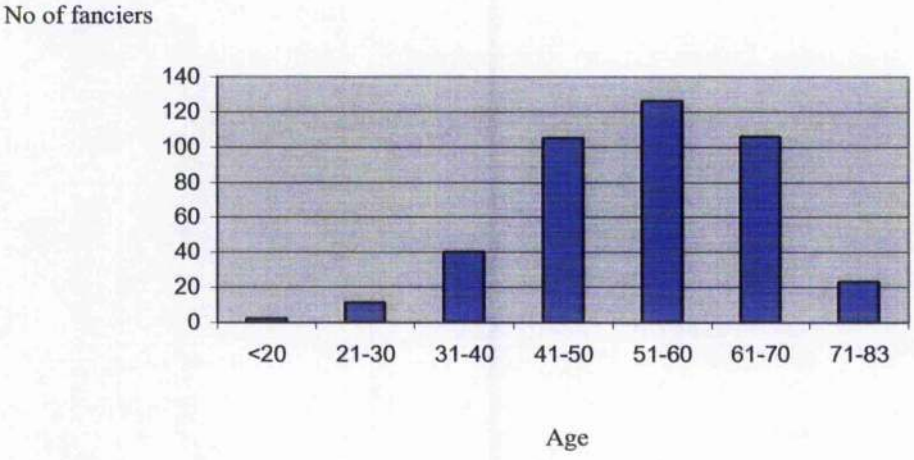

Figure 9. The number of fanciers within each age group

Age range	Number of fanciers	IgG (ug/ml)	Number of years involved
<20	2	1	8
21-30	11	26.8	12
31-40	40	45.3	17
41-50	105	48.7	22
51-60	126	51.5	30
61-70	106	53.6	39
71-83	23	47.6	43

Table 18. Distribution of fanciers according to age and IgG antibody results

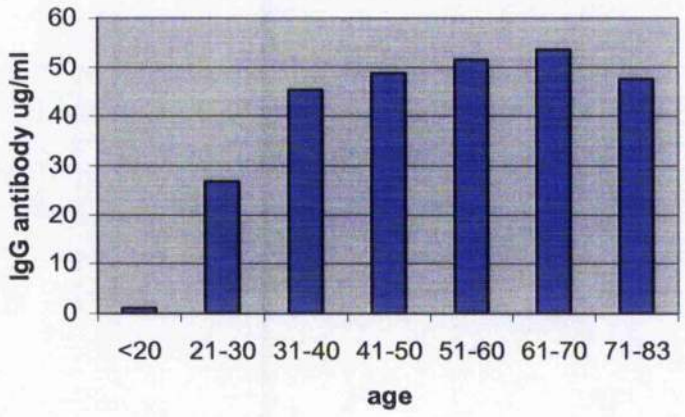

Figure 10. The increase in IgG antibody responses with increasing age group

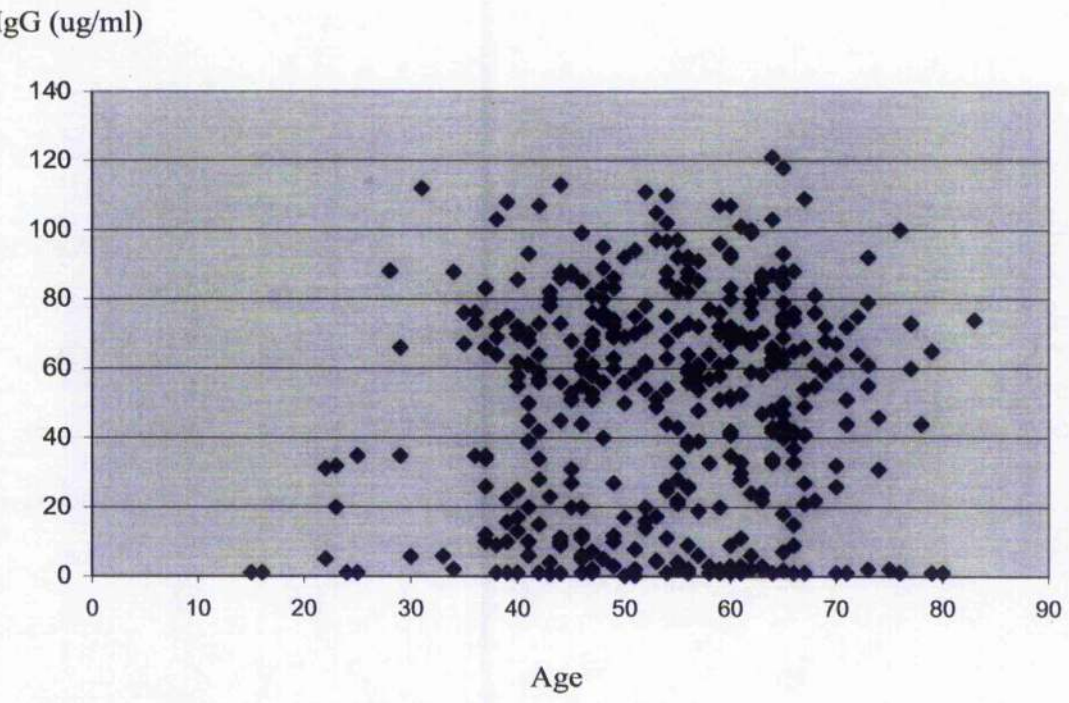

Figure 11. Significant correlation between age and IgG antibody response to avian antigen

No of birds	Non smokers	Smokers	Total of fanciers	Mean IgG(ug/ml) for all fanciers	Mean IgG (ug/ml) for non smokers
25	13	3	16	56.7	62.3
50	24	77	101	51.8	57.5
75	18	68	86	47.4	51.2
100	31	101	132	46.5	52.7
125	3	8	11	58.8	59.2
150	2	18	20	47.2	50.7
175	3	18	21	72.3	77.1
200	2	8	10	51.1	54

Table 19. The IgG antibody results of 397 fanciers including non smokers according to the number of birds kept

It seems that with increasing age and avian exposure, the ability to mount an antibody response increases. However this high level of IgG antibody may not necessarily translate into clinical symptoms allowing these individuals to continue with their hobby. This picture is distorted by the fact that individuals with severe symptoms or those with acute progressive disease will stop avian exposure completely.

iii) Avian exposure

The antibody response to avian antigen is likely to be influenced by the extent of exposure to these relevant antigens. Fink and colleagues (141) proposed an exposure index to include the length of time spent with birds and the number of birds. They suggested that there was a higher incidence of precipitants in the individuals with a greater exposure index. This relationship is reviewed in this section. The IgG antibody levels are measured against several measurable avian antigen exposures including number of birds, length of exposure in one week and number of years involved with the birds in 397 pigeon fanciers.

a) Number of birds kept

Increments of 25 birds were taken as a reasonable scale of the numbers of birds kept in the lofts. The average number of birds kept was 85. Table19 shows the mean IgG results according to the number of birds kept. There was no correlation between the number of birds kept and IgG responses ($r=0.25$, $p=0.55$) even after smoking habit was taken into account ($r=0.15$, $p=0.72$). Overall the non-smoking group had a higher antibody response.

b) Years of avian exposure

The number of years of avian exposure is related to the age of fanciers and apart from the youngest age group the number of birds kept was almost similar (Table 20). There was no significant difference in mean IgG antibody levels between the study groups and no significant correlation between the number of years involved and IgG responses (Table 21, $r= 0.047$, $p= 0.347$).

Number of fanciers	Age range	Years involved	Number of birds
2	<20	8.5	40
11	21-30	11.8	82
40	31-40	16.9	84.5
105	41-50	21.8	87.7
126	51-60	29.6	88.7
106	61-70	39	85.3
23	71-83	43.2	90.3

Table 20. Number of years involved with pigeon fancying and number of birds kept according to age group

Years of involvement	IgG (ug/ml)
<10	46.6
10	51
20	47.8
30	53.4
40	52.8
50	49.2
60	51.6

Table 21. IgG antibody response according to the number of years involved in keeping birds

Weekly hours contact	Number of fanciers	IgG (ug/ml)
0-10	126	50.3
20	141	51.2
30	68	48.6
40	27	49.4
>40	15	47.5

Table 22. IgG response according to the number of hours in a week spent in the lofts

Therefore the increasing number of years with avian antigen exposure does not seem to influence IgG antibody production.

c) Weekly hours of avian antigen contact

The average weekly hours contact with pigeons was 18.4 hours for 377 fanciers surveyed. Table 22 shows the average IgG antibody level for each group divided by the number of hours spent with their pigeons in a week. There was no significant correlation between the number of weekly hours spent with their pigeons and IgG antibody response ($r=-0.02$, $p=0.69$). It would seem that increasing weekly avian contact, like the other measures of increasing avian exposure, does not influence the amount of IgG antibody production. However it is important to emphasis that most fanciers are aware of their symptoms and may self regulate their antigen exposure by reducing their exposure time as a compromise between their symptoms and hobby.

4. The association of serum IgG with diseases

i) Profile of antibody levels

The serum IgG antibody to avian antigen of 424 fanciers were measured and divided into levels of immune sensitisation as in figure 12. The mean IgG level was 49.2 ug/ml for the whole group and the highest level was 121ug/ml. 86 (20%) of the fanciers had little or no evidence of sensitisation. 236 (55%) had IgG levels above 50ug/ml.

ii) Incidence of PFL and association with antibody response

In this section, the number of respiratory and systemic symptoms experienced (Table 23) and IgG antibody levels are reviewed in 422 pigeon fanciers. Fanciers describing at least one systemic and one respiratory symptom related to contact with their pigeon occurring on a regular basis are considered to have probable PFL.158 fanciers (37%) were considered to have probable PFL having fulfilled the criteria above. The incidence of disease was increased from 28% to 42% in fanciers with IgG antibody level above 30ug/ml.

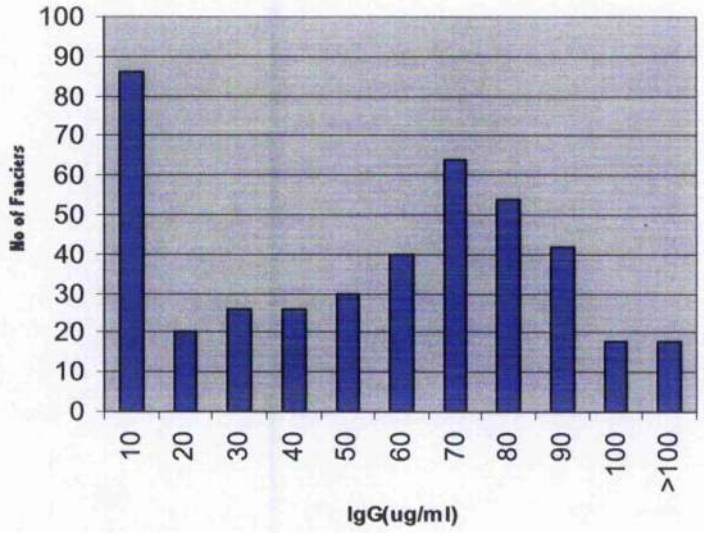

Figure 12. Distribution of fanciers according to IgG responses

Pulmonary symptoms	Systemic symptoms
Dyspnoea	Shivery
Upper respiratory tract symptoms	Fever
Chest tightness	Sweating
Wheezing	Tiredness
Dry cough	Aching muscles
Flu like symptoms	

Table 23. Pulmonary and systemic symptoms complained by pigeon fanciers

Number of fanciers (%)	IgG levels (ug/ml)	No of average symptoms
66(15.6)	0-5	2
44(10.4)	6-20	2.8
81(19.2)	21-50	2.6
138(32.8)	50-75	3
76(18)	75-100	3.8
16(3)	>100	3.4

Table 24. The average number of symptoms complained in each IgG antibody increment group

Of the 422 fanciers studied, 88 fanciers denied having any symptoms whatsoever although their average IgG antibody level in this asymptomatic group was 40 ug/ml. The number of symptoms complained for each antibody increment groups are illustrated in table 24. Fanciers with increasing number of complaints had a higher average IgG antibody level as shown in figure 13 ($r=0.18$, $p=<0.05$). This shows that fanciers with higher IgG antibody levels are likely to become more symptomatic with classical PFL features.

iii) IgG antibody and lung function

64 pigeon fanciers attending a pigeon show conference were involved in this study. The aim of this study was to determine any relationship between antibody response and airflow limitation in pigeon fanciers. Fanciers filled in a self-completed questionnaire, gave 5ml of blood for IgG antibody measurement and performed a lung function test with a spirometry according to the ATS guidelines. The fanciers were divided into 3 groups of symptomatic non smokers, asymptomatic non-smokers and asymptomatic smokers. The results are shown in table 25. There was no significant difference in lung function measured by spirometry in the 3 groups. There was a significant difference in IgG antibody response between smokers and non-smokers where smokers had a much lower level response compared to the two non-smoking groups. However the asymptomatic non-smokers have the highest average IgG levels. There was also no correlation between lung function and IgG response (FEV1 $r=-0.17$ $p=0.18$, FEV1/FVC ratio $r=-0.12$ $p=0.16$, FEF_{25-75} $r=-0.154$ $p=0.22$).

Therefore IgG antibody levels seems to be an unreliable test as an indicator of possible airway limitation in pigeon fanciers but further long term studies are required to determine whether these individuals are more prone to develop airflow limitation in the future if they persistently remain antibody positive This study also showed that asymptomatic non-smokers do develop a significant humoral response and in this group the response is higher than the symptomatic group although in cigarette smokers the responses are suppressed.

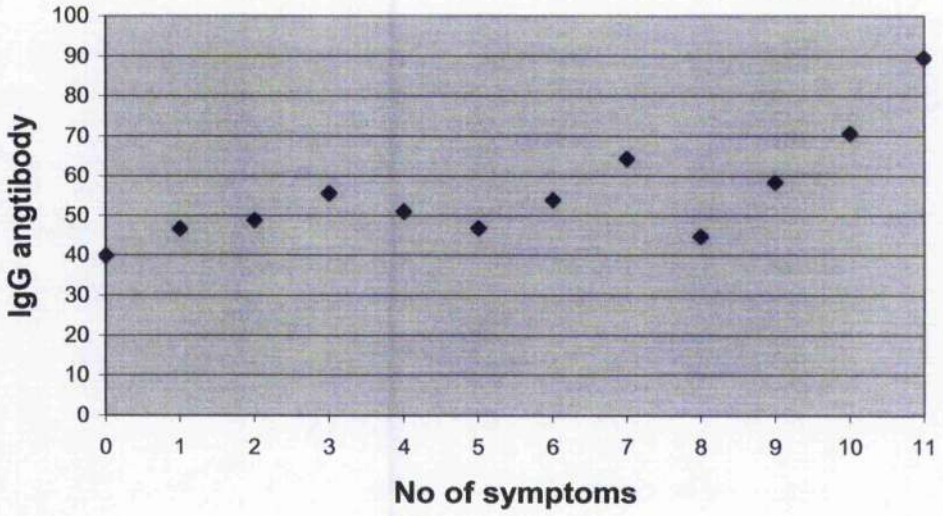

Figure 13. Number of symptoms experienced in relation to IgG antibody response

	Symptomatic	Asymptomatic	Smokers
n	26	22	16
FVC (% meas)	102 (93-109)	107 (100-116)	108(90-124)
FEV1 (% meas)	95 (87-108)	96 (86-105)	97 (82-108)
FEV1/FVC ratio	74.7 (69-84)	71.7 (66-78)	73.8 (70-79)
FEF 25-75 (% meas)	74.5 (48-100)	69.5 (52-95)	71.7 (50-80)
IgG (ug/ml)	49 (18-83)	61.4 (32-86)	18 (3-28)

Table 25. Spirometry and IgG antibody results in 64 volunteer pigeon fanciers in 3 groups

5. DISCUSSION

An important step in the production of antibodies is the antigen specific interaction between a T helper lymphocyte and a B lymphocyte, which leads to their mutual activation. The antibody response to antigens requires the relevant B cell recognising antigen with its surface Ig receptor and receiving certain activation signals from CD4+ T helper cells. T cell recognition of antigens bound to class II MHC molecules on the B cell surface and co-stimulatoy signals lead to T cell activation and secretion of T helper lymphocytes. Genetic factors are likely to control the responsiveness to the antigen, the ability and extent of antibody production and the development of pneumonitis.

As mentioned in the introductory chapter other environmental and constitutional factors may affect the clinical outcome to individuals exposed to avian antigens. In this chapter we reviewed the effects of various parameters including smoking habit, age and avian exposure on the antibody response.

One of the strongest environmental factors affecting the antibody response is cigarette smoking. Pigeon fanciers who smoke have a lower IgG antibody response compared to non-smokers (142,143). Previous studies have also confirmed a lower incidence of farmers' lung and in other causes of HP in non smokers (144-146). This suggests that smoking suppresses humoral response to antigens. The effects do not seem to be permanent as the antibody levels of ex smokers return to similar levels as non-smokers. In the past it was thought that smoking causes airway obstruction resulting in proximal deposition of inhaled antigen preventing access to the alveoli where the disease is most prominent (147). However the global influence of cigarette smoking on the immune system is still not fully understood. It has been shown that smoking increases the alveolar neutrophils and macrophages and changes their physical properties and this may suppress the lymphocyte ratio. Alveolar macrophages play an important role in the regulation and inflammatory responses. Alveolar macrophages also collaborate with lymphocytes in generating specific immune responses, including antigen presentation (148). In cigarette smokers, alveolar macrophages exhibit reduced responsiveness towards liposaccharide (LPS) due to inhibition of production of TNFα and IL-6 resulting in decrease resistance

against foreign invading pathogens. The production of other cytokines such as IL-1 and IL-8 has also been observed (149). Studies in patients with farmers lung have shown that smokers with farmers lung had more frequent illness, more likely to develop insidious than acute symptoms, have worse lung function and had poorer 10 year survival compared to non smokers with farmers lung (150). Although PFL appears to be more common in non smokers, the prognosis may be worse in smokers. Therefore smoking should be strongly discouraged among pigeon fanciers.

With increasing age there was a progressive tendency towards producing an antibody response. In contrast, previous studies showed that increasing age with more years of avian exposure had progressively lower mean antibody level. This was thought to be due to a desensitising mechanism, which has been shown in animal studies involving prolonged exposure to inhaled or intravenous antigens (151-154). This desensitisation is associated with gradual resolution of ganulamatous mononuclear cell infiltrates in the lung. Desensitised animals become refractory to the development of inflammatory responses for more than 2 months and following the refractory period an anemnestic response can be induced with repeated antigen challenge. Experimental studies have also shown that lipo-oxygenase inhibitors and suppressor factors produced from macrophages, NK cells and T cells can modulate and dampen the granulamatous inflammatory lesions in the lung after repeated antigen exposure (154-156).

However, these results from this study need to be interpreted with caution as symptomatic fanciers perform self regulatory measures which reduce the antigen exposure controlling their symptoms and fanciers with severe symptoms may need to stop exposure completely whilst asymptomatic seropositive fanciers carry on with their activities as usual.

This study looking at the role of antigen exposure towards mounting an antibody response showed no clear association. Fink et al (141) found that the presence and intensity of serum precipitants to avian antigens are related to exposure index rather than with symptoms in a survey of 200 pigeon fanciers where none described symptoms

typical of PFL. In this study the antigen exposure measured by the number of birds kept, total weekly hours spent in pigeon lofts and years spent in the sport we did not find a correlation between antibody response and increasing antigen exposure. However the number of symptoms expressed by fanciers correlated with an increase in the antibody response and the incidence of PFL is highest in the most vigorous response. Therefore, in this group of fanciers it may be reasonable to conclude that additional constitutional and environmental factors are more important than the total antigen exposure itself, in determining the antibody response to these inhaled antigens. The level of IgG antibody is determined by the host immunological responses rather than the duration or intensity of antigen exposure.

In this study of a large population of pigeon fanciers the majority of fanciers had evidence of sensitisation (80%) when the IgG antibody was measured by the ELISA technique. This is in contrast with previous studies of HP where the reported incidence of precipitating antibody ranged from 11% to 60% (157,158).

Using this sensitive technique, this study of 412 pigeon fanciers showed a clear association between increase incidences of PFL with progressively higher IgG antibody levels. This IgG antibody response reflects exposure to antigen and the higher response reflects increasing symptoms. Therefore fanciers with high levels of IgG antibody even without any noticeable symptoms should be advised to take precautionary measures to reduce antigen exposure as they may progress to develop the clinical disease. There are fanciers who reported significant symptoms despite little or no evidence of sensitisation. Their symptoms may well be because of other diseases that had developed while being within the loft environment. There are many irritant dusts that are inhaled within the loft and can cause symptoms similar to PFL. The dust in the pigeon lofts have been shown to contain various bacterial and fungal products such as bacterial lipopolysaccharide, fungal beta glucan (21), teichnoic acid and C substance like carbohydrate material (159). Other antigens such as pigeon feather mites can constitute up to 10% of the weight of the feather and storage dust mites and cause clinical symptoms of asthma or rhinitis (160-161). Human ornithosis following contact with pigeons may also occur. Chronic exposure of irritant dusts such as grain dust (162) can lead to the development of chronic

bronchitis (9,10). The influence of these antigenic dusts and the additional descriptions of asthma and rhinitis can complicate matters in making a diagnosis. How important is the exposure to these airborne toxins or allergic substance is unknown and further air sampling studies along with serial measurements of immune response, lung function and clinical status will help in defining some of the clinical symptoms described.

CHAPTER 5

ANTIGEN AND ENDOTOXIN INDUCED CYTOKINES IN PFL

1. Summary

The immune-pathogenesis of pigeon fanciers lung (PFL) is uncertain but avian-antigen specific antibody and lymphocytes appear necessary but not sufficient for disease. Therefore, the involvement of pro-inflammatory and T cell regulatory cytokines were investigated as an additional component required for the expression of disease. Based on a structured questionnaire, 21 pigeon fanciers with classical symptoms of PFL and 27 matched for age and avian exposure with no symptoms and 12 control subjects with no avian exposure were interviewed and blood samples were taken for measurement of IgG antibody and lymphocyte proliferative responses to avian antigen. The proinflammatory and regulatory cytokines stimulated with either antigen or bacterial endotoxin were also measured.

Subjects with classical symptoms of PFL had an increase in avian-antigen specific serum IgG antibody and blood lymphocyte proliferative responses. There was no significant difference in the constitutive or endotoxin-inducible level of acute-phase pro-inflammatory cytokines between the study groups. The symptomatic pigeon fanciers had a dose-dependent increase in antigen-induced TNFα, IL-1β, IL-6, IL-8 and IL-18 and the antigen-induced cytokine levels correlated with the avian-antigen specific serum antibody level.

The production of pro-inflammatory and immuno-regulatory cytokines in response to endotoxin was normal. However there was a dose-dependent antigen-specific production of these cytokines that was significantly associated with susceptibility to disease. The levels of these cytokines correlated with the avian antibody titres. This suggests that the cytokine production mediated by immune complexes was quantitatively and qualitatively different from the cytokine profile produced by endotoxin and may predispose to disease.

2. Introduction

The acute symptoms of PFL include shortness of breath, cough, fever and myalgia suggests both local pulmonary and systemic acute phase reaction. The majority of fanciers do produce an antibody response to avian antigens but many remain without symptoms. The immune-pathogenesis is still unresolved but avian-antigen specific antibody and lymphocytes appears necessary but not sufficient for disease.

Cytokines are signal molecules controlling the state of activation of all local immunocompetent cells, in both hypersensitivity reactions and allergic responses taking place in the lung. There is evidence supporting the paradigm that a complex network of cytokines governs the initiation, maintenance and resolution of an immune response (164-166). Therefore, the involvement of pro-inflammatory and T cell regulatory cytokines may be an additional component required for the expression of disease (167). A number of animal model studies have been performed looking at the role of cytokines in the production of granulamatous pulmonary infiltrates closely resembling human allergic alveolitis. A range of cytokines is produced and the pro inflammatory cytokine TNFα is perhaps the most important in the development of experimental granulomas. The distribution of these cytokines and the genetic polymorphism, which determines the ability to produce the amount of cytokines, may determine the onset of clinical expression and progression of the disease. TNFα, which has been shown to be an important mediator in HP (168-172) in both human and animal studies is mainly produced by alveolar macrophages, plays a critical role in lung injury including the regulation of fibroblast growth via induction of IL-6, release of other lymphokines including IL-1 and IL-6 and increases prostaglandin production (173-175). Antisera against recombinant TNFα have been shown to reduce pulmonary granuloma response (18). Pro inflammatory cytokines such as TNFα , IL-1β, IL-8 and IL-6 may be responsible for the acute phase reaction in PFL causing fever and leucocytosis. Alveolar macrophages also produce T cell regulatory cytokines IL-12, IL-15 and IL-18, which can polarise lymphocytes primarily towards a Th-1-type response (176). In this study we reviewed the role of these pro inflammatory and regulatory cytokines (Tables 26 and 27) in a group of pigeon fanciers.

TNFα - actively produced by lung macrophages. Plays a critical role in lung injury and regulation of fibroblast growth via induction of IL-6. Essential for cell recruitment at sites of inflammation and acts as an early response cytokine in the promotion of a cytokine cascade (164). Releases other lymphokines (IL-1,GM-CSF,IL-6) and increases prostaglandin production. Chronic overexpression of TNFα and IFNγ and the dysregulation of TNF-R(receptor)/TNF-L(ligand) causes persistence and progression of inflammatory pulmonary disease and can lead to chronic recruitment of inflammatory cells, which once in tissues, assemble granulomatous structures.

IL-1 – Produced in response to inflammatory stimuli. Provides accessory growth factor activity for inflammatory lung T cells (165). Stimulates T helper cells, which are induced to secrete IL-2 and to express IL-2 receptors. Regulates the development of alveolitis by promoting the adhesions of neutrophils, monocytes and T cells by enhancing the expression of adhesion molecules such as ICAM-1/CD54 and ELAM/CD62E. Promotes fibroblast proliferation and collagen production and is involved in the development of lung fibrosis.

IL-6- A variety of chronic pulmonary inflammatory diseases including sarcoidosis, tuberculosis and interstitial lung disease associated with autoimmune lung disorders are thought to involve dysregulation of IL-6 production. IL-6 influences antigen specific immune responses, potentiates inflammatory reactions and is also involved in controlling fibroblast proliferation.

IL-8 – Stimulates the accumulation of neutrophils, activated CD8 and monocytes in hypersensitivity pneumonitis

Table 26. Proinflammatory cytokines

IL-12 – Involved in Th1 immune responses and stimulates activated T lung cells and NK cells. Induces Th0 versus Th1 shift and in synergy with IL-15 favours the contact between activated T cells and antigen presenting cells. Acts by interacting with specific receptors (IL-12Rβ) expressed by lymphocytes during most Th1 driven diffuse lung disease.

IL-15 – Involved in the development of T cell alveolitis by supporting the growth and chemotaxis of T cells. It also acts as a co stimulatory factor for production of other cytokines and chemokines (CXCL8/IL-18,GM-CSF,IFNγ and TNFα).

IL-18 – Previously known as IFN-γ-inducing factor and is almost similar in its activity to IL-1. Simulates IFNγ production and causes inflammation by inducing the synthesis of pro inflammatory cytokines TNFα, IL-6, IL-8and IL-1β. The regulatory cytokines IL-18, IL-12 and IL-15 act on Th1 cells synergistically to induce IFNγ towards a Th1 response while inhibiting the production of IL-10.

Table 27. Regulatory cytokines

3. Aims of study

The aim of this study was to investigate whether pigeon breeders with symptoms typical of PFL displayed disproportionate amounts of monocyte-derived pro-inflammatory and T cell regulatory cytokines when stimulated non-specifically with bacterial endotoxin or specifically with avian antigen *in vitro*.

4. Subjects and Methods

60 non-smoking pigeon fanciers attending a National Convention of Pigeon Fanciers volunteered for assessment. The volunteers filled in a self completed questionnaire (Appendix A), performed a spirometry test based on the ATS guidelines on performing spirometry (120) and provided a blood sample (5ml).
Subjects were considered symptomatic if they have at least one classical respiratory and one classical systemic symptom related to contact with their pigeons on a regular basis. Based on the self completed questionnaire (Appendix A), 21 described classical symptoms of PFL and 27 matched for age and avian exposure had no symptoms. Blood samples were taken along with 12 control subjects with no avian exposure.
Serum IgG antibody against avian antigen was quantified by enzyme linked immunoassay ELISA (Appendix B). Lymphocyte proliferative responses to avian antigen were quantified by tritiated thymidine incorporation in 7 day whole blood assay. Supernatant from cultures unstimulated or stimulated with either antigen or endotoxin were harvested after 2 days and cytokine levels were measured by commercial EIA. Statistical analysis was done using Minitab software using paired T test and Pearson correlation coeficient

5. Results

There were no significant differences in the demographics (age) nor the indices of avian exposure between the study groups of pigeon fanciers with and without a history of

symptoms in keeping with PFL. The measures of pulmonary function were lower in those with symptoms suggestive of PFL (Table 28).

Comparison between the study groups of pigeon fanciers with and without a history of symptoms in keeping with PFL demonstrated a more abundant avian-antigen specific serum IgG antibody and blood lymphocyte proliferative responses among those with disease (Table 29).

There was no significant difference in the constitutive or endotoxin inducible level of acute-phase pro-inflammatory cytokines between the study groups. The symptomatic pigeon fanciers had a dose-dependent increase in antigen-induced TNFα, IL-1β, IL-6 and IL-8. The endotoxin-induced levels of TNFα and IL-1β greatly exceeded the antigen-induced levels but in the case of IL-6 and IL-8 the antigen-induced cytokine levels were equivalent to or greater than the endotoxin-induced levels (Table 30).

The avian-antigen specific serum antibody level correlated with the antigen-induced TNFα level ($r=0.36$, $p<0.05$), the IL-1β level ($r=0.45$, $p<0.01$), the IL-6 level ($r=0.6$, $p<0.001$) and the IL-8 level ($r=0.41$, $p<0.01$).

There was no significant difference in the constitutive, endotoxin- and antigen-inducible level of monocyte-derived T cell regulatory cytokines IL-12 and IL-15 between the study groups (Table 31). The symptomatic pigeon fanciers had a dose-dependent increase in antigen-induced IL-18. The endotoxin-induced levels of IL-12 greatly exceeded the antigen-induced levels but in the case of IL-18 the antigen-induced cytokine levels approximated the endotoxin-induced levels.

The antigen-induced IL-18 level correlated with the avian-antigen specific serum antibody level. ($r=0.46$, $p<0.01$).

	Asymptomatic n=27	Symptomatic n=21
Age	53 (43-61)	47 (41-60)
Avian exposure		
Years	20 (19-39)	20 (10-37)
Hours / week	16 (7-25)	14 (7-20)
No of birds	47 (40-65)	60 (50-80)
Pulmonary function		
FVC %	98 (79-114)	90 (77-101)
FEV1 %	94 (78-104)	81 (64-99)
PEF %	110 (93-121)	99 (73-112)
FEF 25-75 %	72 (50-92)	65 (33-101)

Table 28. The demographics, indices of avian exposure and measures of pulmonary function (median and i-q range) among pigeon fanciers with and without a history of symptoms in keeping with PFL

	Asymptomatic n=27	Symptomatic n=21	p value
Immunology			
IgG antibody	44 (9-59)	59 (41-83)	0.03
CMI cpm	109 (89-165)	159 (109-245)	<0.05

Table 29. The avian-antigen specific serum IgG antibody (ug/ml) and lymphocyte proliferative responses (counts per minute) between the study groups of pigeon fanciers with and without a history of symptoms in keeping with PFL

	Controls n=12	Asymptomatic n=27	Symptomatic n=21
TNFα			
Constitutive	95 (34-108)	32 (16-58)	67 (48-112)
Endotoxin	1582 (864-2704)	2340 (1000-3195)	2481 (1560-6556)
Antigen 5 ug	25 (13-101)	28 (7-63)	91 (32-161)
Antigen 50 ug	27 (12-86)	45 (16-402)	529 (77-1481)
IL-1β			
Constitutive	9 (5-23)	8 (4-15)	14 (6-42)
Endotoxin	1184 (848-1697)	1123 (802-1432)	1219 (767-2342)
Antigen 5 ug	4 (0-22)	5 (0-60)	73 (11-177)
Antigen 50 ug	0 (0-9)	53 (0-218)	190 (25-448)
IL-6			
Constitutive	495 (304-1050)	370 (221-715)	675 (142-950)
Endotoxin	6979 (6598-7614)	6521 (5556-7046)	7137 (6140-8094)
Antigen 5 ug	252 (123-422)	608 (161-1940)	2840 (351-6825)
Antigen 50 ug	216 (100-374)	2531 (205-5712)	5340 (279-9533)
IL-8			
Constitutive	2.5 (1.1-5.2)	2.2 (0.8-4.5)	3.6 (1.0-14.4)
Endotoxin	22.3 (19.9-23.7)	23.4 (20.2-26.0)	24.2 (19.5-28.2)
Antigen 5 ug	0.9 (0.3-3.2)	6.0 (1.5-23.8)	64.9 (8.5-210)
Antigen 50 ug	1.2 (0.4-106)	32.6 (6.6-48.8)	38.4 (5.1-186)

Table 30. The endotoxin and avian-antigen inducible acute-phase pro-inflammatory cytokine levels (pg/ml) in monocyte culture from pigeon fanciers and controls.

	Controls n=12	Asymptomatic n=27	Symptomatic n=21
IL-12			
Constitutive	93 (66-149)	71 (42-134)	90 (51-164)
Endotoxin	5774 (4431-6784)	6701 (3528-8832)	5693 (3556-8082)
Antigen 5 ug	90 (54-133)	67 (49-146)	104 (67-163)
Antigen 50 ug	96 (63-140)	121 (72-222)	137 (78-154)
IL-15			
Constitutive	0 (0-0)	0 (0-0)	0 (0-4.5)
Endotoxin	59 (0-148)	12 (0-60)	3 (0-82)
Antigen 5 ug	0 (0-33)	0 (0-8)	0 (0-8)
Antigen 50 ug	0 (0-36)	0 (0-24)	0 (0-1)
IL-18			
Constitutive	65 (52-97)	52 (32-113)	101 (44-254)
Endotoxin	535 (258-791)	479 (282-939)	692 (444-1147)
Antigen 5 ug	37 (12-70)	100 (62-136)	232 (133-394)
Antigen 50 ug	66 (0-120)	246 (123-579)	285 (44-742)

Table 31. The *in vitro* endotoxin and avian-antigen inducible monocyte-derived T cell regulatory cytokine levels (pg/ml) in pigeon fanciers and controls

6. Discussion

A more abundant avian-antigen specific serum IgG antibody and blood lymphocyte proliferative responses were observed among subjects with classical symptoms of PFL. However there is a large overlap between symptomatic and asymptomatic subjects. Many subjects still produce a positive antibody and lymphocyte response for avian antigen despite lack of clinical expression of symptoms. Therefore antibody appears necessary but not sufficient to cause disease. Animal models despite its limitations have shown that immune complex mediated lung inflammation may be mediated by proinflammatory and regulatory cytokines (65,178-180). This study explored the involvement of these cytokines and its association with the disease.

There was no significant difference in the constitutive or endotoxin-inducible level of acute-phase pro-inflammatory cytokines between the study groups. The symptomatic pigeon fanciers had a dose-dependent increase in antigen-induced pro inflammatory and regulatory cytokines TNFα, IL-1β, IL-6, IL-8 and IL-18. This study did not show an increase in IL-12 or IL-15 although previous studies have shown an increase of these cytokines in patients with hypersensitivity pneumonitis (181,182). The antigen-induced cytokine levels correlated with the avian-antigen specific serum antibody level TNFα, IL-1β, IL-6, IL-8 and IL-18. The production of pro-inflammatory and immuno-regulatory cytokines in response to endotoxin was similar between the study groups. However there was a dose-dependent antigen-specific production of these cytokines, which was significantly associated with susceptibility to disease. The levels of these cytokines correlated with the avian antibody titres. This suggests that the cytokine production mediated by immune complexes was quantitatively and qualitatively different from the cytokine profile produced by endotoxin and may predispose to disease.

The early activation of alveolar macrophages by specific antigen/antibody complexes, with release of these proinflammatory cytokines sets the stage for the development of PFL. Activated T cells of the Th 1 phenotype produces IL-2 and interferon gamma, which can lead macrophages to transcribe and release higher amounts of IL-1 and TNFα.

This process probably contributes to the influx of CD 8+ cells seen in the lungs of affected individuals. Other cytokines may play an inhibitory role in the formation of this inflammatory response. Administration of IL-10 appears to down regulate the Th1 responses with reduce numbers of pro inflammatory cells and down regulation of MHC class II, B7 costimulatory molecule and expression on accessory cells (183).

With recent advances in technology, various genetic polymorphisms for production of enzymes and cytokines have been identified. It may be possible that a combination of these genes confer increase or decrease susceptibility to developing a particular disease. Selman et al identified the association of certain polymorphism and the expression of disease. The group found patients with PFL had an increase of the frequency of HLA-DRB1*1305, HLA-DRQB1*0501 and TNF-2 (-308) promoter allele but a decrease of HLA-DQB1*0402 allele (184). The amount of pro inflammatory and regulatory cytokines produced at each stage may determine the onset and progression of disease and this amount is determined by each individual's constitutional factors such as genetic polymorphism and environmental factors such as cigarette smoking or intercurent infections which can have an immunomodulatory effect on lymphocyte function. However, our knowledge regarding the potential mechanisms whereby cytokines function in the various stages of immune responses are derived from in vitro studies that are often difficult to translate into in vivo inflammatory events. The wide range of cytokine and inflammatory response observed under different clinical situation further complicates this. There is obviously a complex process involving an initial immune response to the development of granulomas and fibrosis mediated by immune complexes, pro inflammatory and regulatory cytokines. This response is modulated by other inhibitory cytokines or suppressor factors produced by various cells, which will dampen the response. This area of research is very important to give us further insight in our understanding into the pathogenesis of hypersensitivity pneumonitis.

CHAPTER 6

FEV_1 AMONG PIGEON FANCIERS CORRELATES WITH CD8+ LYMPHOCYTE COUNT

1. Summary

The respiratory symptoms of PFL include shortness of breath due to restriction of gas transfer suggesting predominantly alveoli involvement. However, the known association with bronchial hyper-reactivity and with chronic bronchitis suggests that large airways may also be involved. Studies have suggested that an imbalance between cytotoxic CD8+ lymphocytes and helper CD4+ lymphocytes may contribute to the abnormal inflammatory process in the airways of patients with airflow limitation. This study looked at the correlation between FEV1 and peripheral blood CD8+ lymphocytes among pigeon fanciers. The IgG antibody response and lymphocyte proliferative responses towards avian antigen were also measured.

Pigeon fanciers with classical symptoms of PFL had an increase in IgG antibody and lymphocyte proliferate response to avian antigens and a higher CD8+ lymphocyte count compared to asymptomatic fanciers. The FEV1 of fanciers with PFL correlated significantly with the blood CD8+ lymphocyte count ($r = -0.5$, p 0.002).

It has been demonstrated previously that FEV1 decreases with increasing years of avian exposure. This index of large airway function appears to be associated with lymphocyte phenotype and function. These observations support the hypothesis that large airways are involved, perhaps sub-clinically, in the disease process of PFL and lymphocyte imbalances are important in the pathogenesis of PFL.

2. Introduction

Although the classical pulmonary abnormalities involves mostly at the alveoli level, the presentation of PFL includes involvement of the upper airways. Some fanciers may present with symptoms suggestive of chronic bronchitis as their only symptom (9,10). Responses to pigeon antigens in peripheral blood T cells obtained from patients with PFL have been described (185-191). Recently there has been interest in abnormalities of T lymphocytes subsets in peripheral blood and in BAL fluid in patients with COPD. Patients with COPD have been reported to have an increase in CD8+ T lymphocytes in the airways, which might suggest that these lymphocytes are involved in the pathogenesis of airflow limitation (122,192,193). It has also been shown that in histopathological specimens of patients with COPD there is an increase in T lymphocytes and smooth muscle mass in the peripheral airways suggesting the presence of small airways remodelling in these patients (194).

3. Aims of study

This study was to investigate the association of FEV1 and CD 8+ T lymphocyte peripheral blood count among pigeon fanciers.

4. Subjects and Methods

Similar patients were used from the above study of 21 pigeon fanciers with classical symptoms of PFL and 27 matched for age and avian exposure with no symptoms and 12 control subjects with no avian exposure based on completing a questionnaire (Appendix A). Apart from IgG antibody and lymphocyte proliferative response measured in the above study, oral temperature and lymphocyte phenotype was counted by flow cytometry (BD Ltd).

5. Results

The demographics, indices of avian exposure and measures of pulmonary function among pigeon fanciers according to their history of symptoms in keeping with PFL is as the above study where there were no significant differences in any of these measures between the groups of pigeon fanciers apart from the lower lung function in fanciers with symptoms suggestive of PFL (Table 28).

The pigeon fanciers with probable PFL had a higher oral temperature, and demonstrated a more abundant avian-antigen specific serum IgG antibody, lymphocyte proliferative response and a higher CD8 lymphocyte count (Table 32).
The CD8 lymphocyte count was significantly associated with the FEV1%. (Figure 14).

6. Discussion

The BAL fluid specimens in patients with PFL shows an increase in both CD4+ and CD8+ T cells but the lymphocytosis is mainly a predominance of CD8+ T cells (195) and characterised by Th1 dominant profile. Animal models have also suggested that the disease is characterised by a Th1 type response (85) and there was a predominance of interferon gamma producing T cells (196), which could be further stimulated by addition of recombinant IL-12 and the addition of IL-10 reduced the production of interferon gamma (197).

The CD8+, cytotoxic T-lymphocyte subset has also been implicated in the pathogenesis of airflow limitation in patients with COPD. Among pigeon fanciers with symptoms suggestive of PFL there was an increase in CD8+ cells, and we described an association between FEV_1 and CD8+ lymphocytes in peripheral blood among all the pigeon fanciers. This finding implies that T lymphocytes might play a role in the pathogenesis of airflow obstruction in PFL. This suggests that the cytokines from CD8+ cells, perhaps gamma interferon, may have a role in the tissue remodelling associated with lung function

abnormality. Among the asymptomatic group there is still a significant immune response with increase in IgG and lymphocyte proliferative response. These changes including the rise in CD8+ count among asymptomatic subjects might be a sub-clinical marker for subsequent disease. Increases in T cell response in asymptomatic fanciers have been reported although some differences in the response have been noted (189). It has been reported of apparently antibody negative individual showing lymphocyte transformation in response to pigeon antigens (198). One study have shown that a range of antigens is able to stimulate T cell responses from both symptomatic and asymptomatic exposed fanciers and a 220kDa protein appeared to be immunodominant and preferentially induced responses in patients with PFL (199).

T cell lymphocytes are clearly vital in the pathological immune response in PFL. It may be the differences in the T cell responses of each individual fancier that will determine the pattern of cytokine production, which in turn will provide protection or stimulation for progression of the immune response and subsequent disease (166,200).

	Asymptomatic n=27	Symptomatic n=21	p value
IgG antibody	44 (9-59)	59 (41-83)	0.03
CMI cpm	109 (89-165)	159 (109-245)	<0.05
Temperature	36.2 (35.6-36.5)	36.6 (36.3-37.0)	0.007
CD8 (cells/cmm)	1232 (913-1540)	1568 (1122-1992)	0.03

Table 32. The avian-antigen specific serum IgG antibody (ug/ml), lymphocyte proliferative responses (counts per minute), oral temperature and CD8+ lymphocyte count between the study groups of pigeon fanciers with and without a history of symptoms in keeping with PFL.

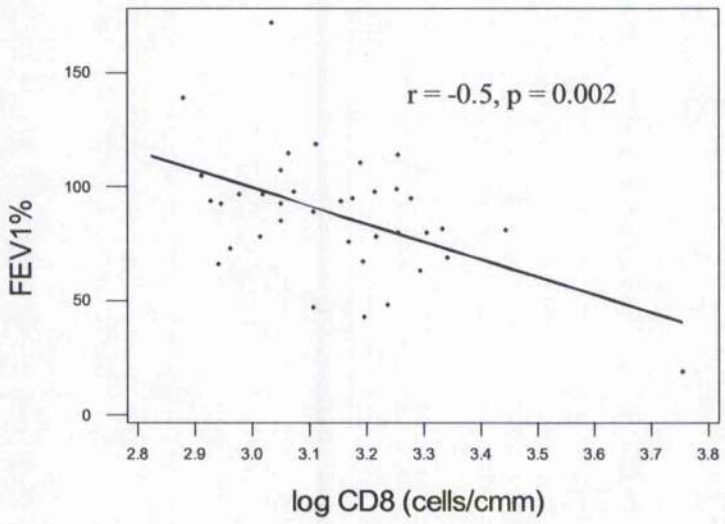

Figure14. Association between CD8+ and FEV1%

CONCLUSIONS

Animal tends to tolerate inhaled antigens and develop immunological tolerance but in humans these antigen induce a powerful immune response in the lung often with clinical symptoms and development of diseases such as asthma, occupational lung disease and HP. The natural history of Pigeon Fanciers Lung is still partially understood and requires further study. In PFL there is an exaggerated immune response against inhaled avian antigen. The level of this response measured by serum IgG antibody against avian antigen reflects the degree of local inflammation in the lungs. The persistence of antigenic stimulation and ongoing chronic inflammatory response leads to the development of granuloma or the development towards fibrosis.

Although the majority of fanciers develop antibody response, only a small number progress to develop established disease. The prognosis seems to be very good in patients with acute non progressive recurrent disease where some adjustment to reduce antigen exposure is sufficient to control their symptoms within a few weeks or months and they are able to continue with their involvement with their pigeons. However further long term studies are required to answer the question of whether fanciers with subclinical disease progress to develop establish disease. In some fanciers symptoms are non specific until they present to medical staff with the established chronic form of the disease and the progression of disease may continue despite avoidance of antigen. There is evidence that the long-term prognosis of farmer's lung is poor where some patients continue to progress despite avoidance of antigen exposure and there seems to be several predictors of long term decline these farmers (55). Weather these prognostic indicators are similar to PFL requires further investigation. Patients in the chronic stage tend to slowly progress with irreversible lung damage leading to respiratory failure, cor pulmonale and ultimately death.

The 5-year longitudinal study among a cohort of pigeon fanciers showed deterioration in lung function over that period. However these changes did not translate into clinical symptoms. This is complicated by the fact that the majority of fanciers who remain active have good lung function and perform self regulatory measures when they develop

symptoms. Fanciers with severe symptoms with progressive disease and poor lung function would not have any choice but to give up their hobby. This study did not demonstrate any relationship between IgG antibody response to avian antigen and FEV1. Therefore, the magnitude of antibody response does not predict airflow limitation in pigeon fanciers at least in the short term. There had been studies showing that the severity of airflow limitation in patients with COPD is associated with airway inflammation and an increased proportion in B cells in the airway wall and reduced proportions of blood B cells suggesting a migration to the site of airway inflammation (201,202). There was a relationship between IgG antibody response to avian antigens and imbalance of T cell lymphocytes. The ratio of these CD4+ and CD8+ T cell lymphocytes correlated with lung function of these fanciers suggesting this imbalance of T lymphocytes may play a role in airway inflammation and obstruction. This study showed no significant changes in symptoms reported over the 5 year period suggesting a state of equilibrium has been reached between the host and external antigen despite continued exposure and persistent antibody production. However the concern remains that this persistent antibody response underlies continuing immune activation and the absent of clinical symptoms may mask the progression to the chronic insidious form of the disease (203-205).

The trend of immune sensitisation over a 13-year period showed a shift in the level of sensitisation among pigeon fanciers in the United Kingdom. This survey also showed that the majority of active fanciers showed evidence of antibody response and the symptomatic fanciers had the highest response. There remains a large overlap of antibody levels between symptomatic and asymptomatic fanciers which strengthens the argument that antibody is necessary but not sufficient to cause the disease.

It has been shown that the quantitative antibody measurement by ELISA is an important step in making the diagnosis of PFL and is a useful tool in managing the disease. The presence of IgG avian antibody implies exposure to antigen and serial measurements of antibody response with clinical history and radiological appearance would indicate how successful was the antigen avoidance measures that has taken place. The serum IgG antibody response to avian antigen in a population of pigeon fanciers has demonstrated a

range of responsiveness. 20% of fanciers showed no evidence of sensitisation despite being exposed to similar amount of antigen. The reason for this unresponsiveness, whether immunological tolerance, desensitisation or underlying genetic predisposition is uncertain.

There are various constitutional and environmental factors that will influence the susceptibility of an individual to produce IgG antibody response and the development of PFL. In this study we examined several of these factors and their influence in producing an antibody response. Cigarette smokers produce lower concentrations of serum IgG antibody and show a reduced prevalence of antibody positive fanciers. The mechanism for this inhibition and the cell biology of this protective effect is not fully understood. Smoking generally suppresses the humoral response and disrupts the alveoli macrophages functioning. This effect is reversible since the antibody levels in ex smokers are similar to non smokers. Despite PFL being more common in non smokers, the prognosis may be worse in smokers as it has been suggested they may develop the insidious chronic form of the disease. A study by Yoshio et al in Japan showed that there was a high smoking rate in chronic HP cases (110,206,207). Since smoking has been shown to inhibit the production of antibodies, it appears to prevent fanciers from developing the acute onset type of the disease, which would have encouraged them to reduce antigen exposure. Therefore smoking should be strongly discouraged among pigeon fanciers (208).

In the large group of pigeon fanciers that was studied, with increasing age the ability to produce more antibodies also increases. These individuals are in the state of equilibrium where they are able to continue with their hobby with some alteration to their antigen exposure and are able to keep any symptoms under control. When the amount of antigen exposure was measured by number of birds kept, number of years involved and number of hours spent in pigeon loft in a week there was no correlation with the magnitude of IgG antibody response. This suggests in this group of population where they counter a high intensity antigen exposure on an intermittent basis, other factors are more important in influencing antibody production to avian antigen.

The symptomatic fanciers tend to produce a higher antibody response and the higher the antibody response the more likely of developing the disease. Fanciers with IgG level of above 50ug/ml almost certainly suffer from symptoms and would have to perform some self regulation manoeuvre to control their symptoms. There are fanciers who reported significant symptoms despite little or no evidence of antibody response to avian antigen. This may be because of other airborne antigens such as endotoxins that are present in the loft environment, which may cause wheeze and cough after acute exposure and symptoms of chronic bronchitis after chronic exposure. Bacterial and fungal materials that are present in the loft can also cause symptoms that are similar to PFL.

Another constitutional factor that may influence the susceptibility of developing the disease is the distribution of cytokines and the genetic polymorphism that controls the production that may determine the onset of symptoms and disease. This study looked at the involvement of pro-inflammatory and T cell regulatory cytokines as an additional component required for the expression of disease. Symptomatic fanciers produced significantly higher levels of proinflammatory cytokines TNFα, IL-1β, IL-6, IL-8 and regulatory cytokine IL-18. This suggests that cytokines may play an important role in the pathogenesis of PFL. The symptomatic fanciers showed a higher level of IgG antibody, lymphocyte proliferation count and oral temperature. However there was a large overlap in immune response between the asymptomatic and symptomatic group. Studies looking at the interaction between these pro inflammatory cytokines and inhibitory cytokines and their effect in the development of pulmonary inflammation and progression of disease may have important clinical and therapeutic consequences.

There was also a raised antibody response in the asymptomatic group suggesting underlying subclinical inflammation with a raised lymphocyte proliferation count and CD8+ count which may be a marker for progression to disease. The CD8+ count was also correlated to the FEV1 measured, which suggests cytokines from CD8+ lymphocytes perhaps gamma interferon or the imbalance of T lymphocytes itself may be involved in the development of airflow obstruction.

Subclinical disease is common among pigeon fanciers and the evidence suggests there is ongoing inflammation in the individual host with an increase in immune and inflammatory response in the lungs and also in peripheral blood. A raise in the antibody response to avian antigen in asymptomatic subjects are associated with an increase of CRP, gammaglobulins, positive rheumatoid factor, total IgG and KL-6 levels, which indicates local lung injury (209). Therefore antibody positive fanciers should be counselled by the healthcare professionals with care and sensitivity to take precaution to reduce antigen exposure. Symptomatic pigeon fanciers would only contemplate giving up their pigeons only as a last resort after all the other measures are tried and found to be ineffective.

The pathogenesis and the natural history of PFL are only partially understood and many of the details of the cellular processes that are involved in the pathogenesis are still obscure (210). Most of this information is acquired from animal models, study of bronchoalveolar lavage fluids and biopsy samples from patients. The understanding of this immunological mechanism will provide an insight of the pathogenesis of the disease, development and targets in treatment of other causes of HP and interstitial lung disease as the immunological process are similar.

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APPENDIX A

SELF COMPLETED QUESTIONNAIRE

This questionnaire was based on the MRC questionnaire for chronic bronchitis and was modified to include details of avian exposure, symptoms of Pigeon Fanciers Lung, general respiratory symptoms and the fanciers past medical history, occupational and smoking details

Antigen Exposure:

1. Do you keep pigeons?
2. How long have you kept pigeons?
3. How many pigeons do you generally keep?
4. Have you been in contact with other species of birds?
5. If yes what species?
6. During the season how many hours per week do you spent with your pigeons?
7. Do you wear a mask when with your pigeons?
8. If yes, does it help with your chest?
9. Have you been diagnosed as having Pigeon Lung?
10. If so, who is treating you?

Symptom data:

Are you aware of any of the following symptoms when you are, or have been, with your pigeons? Some of this can occur 2-3 hours after you have been with your birds and after a show or a marking session. This may also occur when you have been cleaning out, or after a prolonged time in contact with your pigeon.

11. Shortness of breath?
12. Sneezing, blocked or runny nose?
13. Itchy or running eyes?
14. Tightness in the chest?
15. Shivery/cold feelings?
16. Fever/temperature?

17. Sweating?
18. Wheezing?
19. A dry cough?
20. Tiredness?
21. Aching muscles?

22. Have you noticed "Flu Like" symptoms in the evening or at night which have gone away in the morning?
23. If you have suffered from any of the previous symptoms, are they worse:
    At the weekend?
    After a pigeon show?
    After cleaning out?
24. Have you had any of the above symptoms over the last week?

Respiratory data:

25. Do you have a cough most days of the year?
26. Do you bring up any spit or phlegm?
27. Do you ever get short of breath when walking with others of your own age?
28. Were you chesty as a child?
29. Have you ever suffered from
    Asthma/Hay fever/Dermatitis
    Heart trouble
    Tuberculosis
    Bronchitis
    Asthma
    Pneumonia
    Other chest diseases
30. Have you ever been exposed to coal, silica, asbestos or hardwood dust?
31. Do you, or have you ever smoked?

32. If the answer is yes

No of cigarette/day

No of years

33. If you used to, how many years ago did you stop?

APPENDIX B

Enzyme linked immunoassay

Serum antibody activity against pigeon serum gamma-globulin [PGG] antigen (40% saturated ammonium sulphate fraction), which is distinct from antigens in dietary hens' eggs [16], was measured by indirect enzyme-immunoassay (EIA) and optimised along with test sample dilutions by checkerboard analysis. Briefly, the assay involved coating 96-well polystyrene microtitre EIA plates (Dynatech Ltd, UK) with the antigen at 10mg/L in bicarbonate buffer (0.02M, pH 9.6), at 100ul/well overnight at 4°C. The plates were washed three times in detergent buffer (phosphate buffered saline, 0.02M, pH7.4, containing 0.05% Tween-20, PBS-T). Serum samples were diluted optimally at 1:200 with PBS-T and incubated in duplicate on the same plates at 100ul/well for 1 hour at room temperature, after which the plates were washed as above. Bound antibody was quantified using alkaline phosphatase conjugated anti-human IgG at dilutions recommended by the manufacturers (The Binding Site UK Ltd). After washing as before, the plates were incubated with the colourless substrate p-nitrophenyl phosphate (Sigma UK Ltd) at 1mg/ml in 10% diethanolamine, pH10.5, which is converted by the phosphatase to a yellow product with an absorbance at 405nm. The color (optical density) was measured by spectrophotometer (Dynatech Ltd UK) developed after approximately 30 min; the timing was approximate; allowing a reasonable distribution of color. The E_{405nm} was proportional to the antibody activity if all other reagents are in excess. The specificity of the assay was established by inhibiting the antibody activity with excess free antigen before assay. The assay was unaffected by high immunoglobulin levels because there was no specific activity demonstrable in myeloma

sera. The IgG antibody activity could be quantified by interpolation, using an optical density standard curve from serial dilutions of a standard serum which had been titrated by quantitative precipitation [15]. There were no equivalent standards for the other serum antibody isotypes, nor for antibody activity against other avian antigens, therefore the optical density units were used as a relative measure for the antibody activity.

APPENDIX C

Publications

1. T Ismail, K Anderson, C McSharry, G Boyd. Antigen exposure and IgG antibody response in Pigeon Fanciers. Eur Respir J 2005 ;26: suppl. 49, 374s

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