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Takes your breath away – the immunology of allergic alveolitis

C. MCSHARRY, K. ANDERSON*, S. J. BOURKE† & G. BOYD‡ *Departments of Immunology and ‡Respiratory Medicine, North Glasgow Hospitals University NHS Trust, Glasgow, *Department of Medicine, Crosshouse Hospital, Ayrshire and †Department of Respiratory Medicine, Royal Victoria Infirmary Newcastle upon Tyne, UK*

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SUMMARY

Extrinsic allergic alveolitis (synonym: hypersensitivity pneumonitis) is caused by inhaling antigenic aerosols which induce hypersensitivity responses in susceptible individuals. It is an interstitial inflammatory disease affecting the distal, gas-exchanging parts of the lung, in contrast to allergic asthma where the inflammation is more proximal, affecting the conducting airways. The aims of this review are to describe current concepts of the immunology of this model of lung inflammation, to describe some of the constitutional and environmental characteristics which affect disease susceptibility and development, and to describe topics for prospective study.

Keywords alveolitis farmer's lung lung inflammation pigeon fancier's lung pneumonitis

DEFINITION AND SCOPE OF THE DISEASE

Extrinsic allergic alveolitis (EAA) is found worldwide with many sources of antigen and an associated diversity of names describing the idiosyncratic syndromes (Table 1). The most commonly studied are bird (pigeon) fancier's disease and farmer's lung, and the immunology of these will form the basis of this review. Most syndromes are occupation-related and symptom prevalence can be up to 16% of exposed subjects, with significant morbidity [1–3]. In addition to the clinical and economic importance, the disease has considerable research potential as a model of inflammatory lung disease in which the populations at risk can be identified, the relevant antigens purified, and the immunological and clinical consequences of antigen exposure can be monitored. Many of the pathological characteristics of EAA overlap with other lung diseases and may therefore provide a means to identify common mechanisms of pathogenesis.

THE DIFFERENT STAGES OF DISEASE AND THEIR CHARACTERISTICS

The characteristic acute symptoms of EAA are described as 'flu-like', and these occur several hours after exposure to antigenic dusts so that the temporal association and significance are often not recognized. Despite antigen being inhaled the disease is not restricted to the lung. Both chest and systemic symptoms are evident: mainly shortness of breath and an unproductive cough, along with fever and muscle pain. The disease may present in an insidious chronic form which is characterized by progressive shortness of breath, chronic cough and reduction in exercise tolerance over time. Disease can be dynamic, depending on changing antigen exposure; for example, there is a seasonal expression of EAA in winter when hay harbouring the spores of thermophilic bacteria is dispersed causing farmer's lung, and when pigeons moult in autumn, shedding the feather dust (bloom): a major cause of pigeon fancier's disease. The intermittent nature of antigen exposure means that most individuals have long asymptomatic periods.

The diagnosis of EAA is usually established from a combination of clinical features and investigations (Table 2), which can vary in accordance with the stage of the disease and the time since the last antigen exposure and the amount of antigen inhaled. These reflect the degree of lung inflammation, including reversibility after intervention. The most sensitive methods can demonstrate inflammatory changes in some asymptomatic subjects, usually those with evidence of immunological sensitization, thus confirming underlying subclinical disease [8,9]. Without the warning of symptoms and consequent modification of antigen exposure, it seems that subclinical disease may progress to the chronic, non-reversible form. Thus EAA may allow the study of the immunological changes associated with progression from acute–resolving to chronic–persistent inflammation.

EAA is characterized by an interstitial and alveolar inflammation [13]. The nature of the inflammatory infiltrate reflects the stage of disease. Sampling of airway cells and fluid by bronchoalveolar lavage (BAL) shortly after antigen exposure demonstrates an acute neutrophil infiltrate [14]. This infiltrate becomes predominantly lymphocytic after 24 h [15]. In acute disease these lymphocytes are predominantly CD4 T-cells but in more chronic disease CD8 T cells predominate, along with variable numbers of

Correspondence: Dr C. McSharry, Department of Immunology and Bacteriology, Western Infirmary, Glasgow G11 6NT, Scotland, UK.
E-mail: cms4q@clinmed.gla.ac.uk

Table 1. Common syndromes and causes of allergic alveolitis

A. Farmer's lung	Economically important, common in temperate climates, caused by thermoactinomycetes growing on damp stored hay; *Saccharopolyspora rectivirgula* (*Micropolyspora faeni*), and *Aspergillus umbrosis*
B. Bird fancier's (breeder's) lung	Caused by inhaling transuded serum and secretory proteins on feather dust (bloom) and in bird droppings
C. Summer-type pneumonitis	Seasonal growth of domestic fungus *Trichosporon* sp. mostly in Japan
D. Humidifier fever	Caused by antigens dispersed by contaminated water from re-circulating air conditioning systems which harbour the protozoan *Naegleria gruberi*
E. Isocyanate alveolitis	An example of occupation exposure to a bio-active inorganic molecule which can cause EAA [4].

Table 2. Characteristic features of allergic alveolitis

Clinical signs	Bilateral lung crackles
Chest radiograph	Pulmonary infiltrates [5]
CT scan	HRCT can identify features such as ground glass shadowing and air trapping even when the X-ray is normal [6,10]
Gallium scan	Uptake reflects alveolar macrophage activation [7]
TcDTPA lung clearance	Indicates increased alveolar permeability and subclinical inflammation [8,9]
Lung function	Restriction of lung volumes [11], impaired gas diffusion, hypoxaemia and airways obstruction [12]
Systemic signs	Temperature and neutrophil leucocytosis in acute exacerbation
Bronchial lavage	Initial neutrophil alveolitis followed by lymphocytic alveolitis
Lung biopsy	Lymphocyte infiltrate, granuloma formation, fibrosis

plasma cells [16,17]. There is general mobilization of the immune apparatus of the lung. Bronchus-associated lymphoid tissue (BALT), which is difficult to detect in normal lung, is induced in EAA and is probably maintained by persistent inflammation. This lymphocyte-rich tissue contains discrete tightly packed aggregates or follicles of B-lymphocytes in a network of dendritic cells, with predominantly T-lymphocytic parafollicular areas. The expansion and maturation of a local humoral response can be demonstrated by the expression of the nuclear proliferation antigen Ki-67 on centroblasts located in BALT germinal centres [18]. More advanced disease is characterized by increasing fibrosis with thickening of the alveolar septa and basement membrane [19]. This fibrosis appears to be related to the proportion of activated neutrophils [20]. There are parallel increases in the numbers of mast cells and eosinophils which, with the other inflammatory cells, normalize with time since the last antigen exposure [21,22].

The characteristic feature of EAA is the marked lymphocytic infiltrate, and the proportion of lymphocytes recovered by BAL correlates with the degree of parenchymal inflammation [23]. The selective recruitment and retention of these lymphocytes in the lung by chemokines and adhesion molecules is therefore important. The expression of a dendritic cell-derived CC chemokine 1 (CK1)/CCL18, putatively involved in naive T cell recruitment, is significantly increased in lung biopsy of EAA patients, and there is a correlation between the levels of tissue CCL18 and the number of lymphocytes in the BAL fluid [24]. The lymphocytes for their part have an increased expression of the $\alpha E\beta 7$ integrin [25]. The BAL lymphocytes in EAA have an increased expression of B7, and CD28 and CTLA4 molecules, and the expression of the corresponding B7-co-stimulatory molecules (CD80, CD86) is up-regulated on the alveolar macrophages of EAA patients [26].

THE DIFFERENT COMPONENTS IMPLICATED IN IMMUNOPATHOGENESIS

Antigens

The sources of antigen causing EAA are diverse (Table 1). However, despite this range, the clinical presentation of the different syndromes is similar. This suggests that although the antigen is specific for each syndrome, the subsequent hypersensitivity reactions are common. For this simple reason it could be argued that there can be no disease-specific antigen. There are however, some non-specific factors common to the alveolitis-associated antigens. They are generally derived from organic material, or from organisms growing on organic waste [27]. They are about one micron in diameter which is the correct aerodynamic size to reach and deposit soluble antigen in the alveoli [28], where the disease process is most evident [29,30]. These particles generally have adjuvant activity [31]. This may be relevant because initiation of experimental alveolitis in many animal models, discussed below, is dependent upon adjuvant costimulation with antigen [32].

Although these non-specific activities may have importance in determining the extent of the specific response, all alveolitis-inducing dusts contain numerous antigens. For example, in farmer's lung 46 antigens from *Micropolyspora faeni*, and in bird (pigeon) fancier's disease, multiple antigens have been extracted from pigeon droppings, feathers, serum, egg yolk and white, crop fluid and gut wall. By cross-absorption, the major antigens were demonstrated in the gamma-globulin fraction of pigeon serum and these had immunological identity with IgA in droppings and on the dust extruded from feathers called 'bloom'. This consists of inert keratin particles one micron in diameter which carry serum proteins [33].

Some of the antigens of EAA were considered to be disease-specific because they would precipitate only with sera from patients with EAA [34,35]. These antigens tend to be minor, and the significance of this observation may be overstated. Given the diversity of antigens [36] and the spectrum of symptoms of EAA [37], it is unlikely that just one antigen is responsible. A more likely situation is that the immuno-dominant antigens (depending on their concentration and solubility) generate high antibody titres which are quantitatively greater in those with EAA but still detectable in some asymptomatic subjects. The minor, putative

disease-specific antigens generate a relatively minor antibody response which may be detectable in EAA patients but below the detection limit in symptom-free subjects.

Antibodies

The late onset of symptoms, about 4–8 h after antigen inhalation, suggests that the disease mechanism may resemble the Arthus-type skin reaction following intradermal skin-tests which has the same kinetics. An antibody-dependent inflammatory process has been suggested for both. The Arthus skin histology demonstrates deposition of immune complexes and complement with an influx of neutrophils, similar to lung histology of biopsies taken during the acute phase [14]. Generally biopsies are taken from patients with more long-standing lung disease and show a lymphocytic infiltrate, with minimal evidence for complexes or complement. Therefore the pathogenesis of EAA is thought to be a hypersensitivity reaction against inhaled antigens with involvement of both humoral and cellular immune responses [37].

EAA among bird fanciers, farmers and aspergillosis patients is associated with high titre serum antibody specific to each antigen, and disease in seronegative subjects is conspicuous by its rarity. Antibody can also be detected in asymptomatic subjects therefore the presence of antibody is not sufficient to establish a diagnosis of EAA. More recently, we have observed raised inflammatory markers TNF-α, CRP and total IgG in asymptomatic pigeon fanciers who were seropositive. This and previous observations of physiological changes in asymptomatic seropositive subjects [8,9] suggest that antibody may have an important role in pathogenesis. We have determined that the titre of IgG antibody is associated with a spectrum of symptom severity, including subclinical disease [38] and structural lung damage [39]. To improve national quality control for measuring this antibody, quantitative methods allowing for standardized procedures have been recommended [40]. To promote this initiative, standard sera and antigen preparations for investigating pigeon fancier's allergic alveolitis are available on request from the author.

Quantifying the antibody response to inhaled antigens has demonstrated some interspecies differences. In animal models of experimental lung disease the antibody response to aerosolized protein antigens, for example ovalbumin, is weak and transient, followed by marked immune-tolerance to further stimulation unless boosted by an immunological adjuvant. Humans generally have a rapid, sustained and strong antibody response to new inhaled antigens in occupational or domestic settings. This can be exuberant, with levels approaching milligram levels per millilitre of blood in subjects with even relatively short duration of antigen exposure. This may be of particular importance for example in the case of children exposed to pigeons and who can develop exceptionally high antibody titres and florid disease, both of which decline following removal of the antigen source. In general, continued exposure to high levels of antigen exposure in pigeon fanciers (number of pigeons, hours per day, number of years) is associated with a gradual decrease in titre [39]. A major factor affecting the antibody response is cigarette smoking; antibody production to inhaled antigens is efficiently inhibited in smokers and is reversible in ex-smokers [41].

In order to establish whether there was a compartmentalized antibody response within the lung of pigeon fanciers with EAA, airway fluid sampled by bronchial lavage (BALF) demonstrated significantly raised total protein and immunoglobulin levels and antibody activity which was relatively higher than in serum when compared with albumin levels, suggesting local production [42]. Antibody activity has been described in the IgG, IgA1 and IgA2 isotypes in serum, BALF and saliva [43–47]. The IgG antibody activity in each compartment correlated [42–44], suggesting that measurement in serum may be an appropriate substitute for local lung levels avoiding invasive bronchial lavage, and saliva sampling may be more convenient for epidemiological studies and for monitoring sequential changes.

Macrophages

Alveolar macrophages appear to have a dynamic role in EAA. Two characteristic but non-specific histological features of EAA are the abundant 'foamy' cholesterol-rich macrophages [48], and the macrophage/epithelioid cells surrounding the non-caseating granulomata [49]. The cell turnover of the alveolar macrophage population appears increased; the numbers retrieved by BAL are increased with an increased population of immature $CD14^+$ cells and an associated increase in serum soluble sCD14 [50]. CD14, the LPS recognition molecule, is a monocyte marker which is lost rapidly as these cells traverse endothelium, therefore these observations may reflect an increased influx of monocytes into the inflammatory site [51]. This is associated with increased expression of the integrin adhesion molecules CD11/CD18 and ICAM-1, with associated increases in soluble ICAM-1 in BAL fluid and serum [52,53]. The soluble ICAM-1 level correlated with physiological indices of lung inflammation, including the alveolar/arterial O_2 partial-pressure difference and the diffusing capacity for carbon monoxide [52], all of which normalized after antigen avoidance [54]. These data suggest that the activation status of the lung macrophage may determine the associated lymphocyte recruitment and activation and together modulate the lung inflammation and physiological response to inhaled antigens.

Lymphocytes

Among asymptomatic pigeon fanciers there is a normal phenotypic distribution. However, in acute symptomatic sero-positive subjects, in addition to a neutrophil leucocytosis, there is a CD4 and NK cell lymphocytosis, and in chronic EAA there is a CD8 and NK cell lymphocytosis [55]. There is a lack of standard methodology for investigating antigen-driven proliferative responses in EAA [55]. In general, our unpublished work shows significantly greater than normal lymphocyte proliferative responses to avian antigens only in seropositive pigeon fanciers, with even higher responses in those who are symptomatic. A recent observation of a 21-kDa protein component of pigeon dropping extract with specificity for antibody recognition and lymphocyte proliferation in pigeon fanciers EAA may help clarify the lymphocyte contribution to disease [35]. This had a 57% identity to a *Saccharomyces cerevisiae* chromosome X reading frame, and a peptide synthesized on the basis of the N-terminal sequence of the native peptide had similar antigenic activity. More general availability of this peptide may help identify the phenotype and function of the cells involved in EAA.

Cytokines

The principal immunological co-factors which may offer an insight into the pathogenesis of EAA are the cytokines. These signal molecules regulate the immune, inflammatory and tissue repair mechanisms. The distribution of pro- and anti-inflammatory cytokines and the genetic polymorphism which

determines the ability to produce high or low amounts after stimulation are thought likely to determine the onset and progression of EAA [56]. The acute symptoms, pyrexia and leucocytosis associated with EAA suggest proinflammatory cytokine activity. Using IL-1 as a prototype cytokine, the levels in BAL fluid are increased [17], and alveolar macrophages from EAA patients produce higher constitutive, but lower LPS-inducible levels *in vitro* [57]. This suggests deactivation *in vivo*. The genetic predisposition for cytokine production by gene polymorphism may be involved in EAA. The frequency of the TNFα2 (308) allele, a genotype associated with high TNF-alpha production *in vitro*, was significantly higher in EAA in farmers [58] and pigeon fanciers [59]. TNFα and IL-1β may account for the acute events in EAA, but the accumulation of neutrophils, activated CD8 T cells and monocytes in EAA may be explained by increased IL-8 and MCP-1 which are chemotactic for these target cells in BAL fluid [60,61]. Steroid treatment which prevents cytokine gene transcription resulted in normalization of this pattern.

EAA in most subjects is intermittent, a clear example of which is the periodicity of humidifier fever during which symptoms occur only on the first day back at work (Monday fever) despite similar antigen exposure throughout the week [62]. This attenuation of symptoms after antigen exposure or tachyphylaxis may be cytokine mediated. For example, TNF-receptor expression occurs downstream of TNF induction, and in EAA there are increased levels of soluble TNF receptors in BAL and serum which can block and counter-regulate a preceding TNF proinflammatory response [63]. Cytokines potentially involved in the attenuation of symptoms of acute EAA, despite ongoing antigen exposure, remain to be described further and we have unpublished data to suggest that IL-1 receptor antagonist levels are important in this regard. These may be important because they may mask overt symptoms while allowing other cytokines to mediate the ongoing subclinical lung tissue remodelling and fibrosis associated with long-term antigen exposure.

Alveolar macrophages produce the T cell regulatory cytokines IL-12, IL-15 and IL-18, which can polarize lymphocytes primarily towards a Th1-type response. This area of research is currently very important in clarifying the pathogenesis of EAA. The BAL lymphocytes in EAA have a Th1-type bias [64], characterized by a predominance of IFN-γ-producing T cells, resulting perhaps from a reduction in IL-10 production, and an increase in expression of high affinity IL-12R. In support of this, alveolar macrophages from EAA patients released increased levels of IL-15 [65], and our unpublished data suggest that IL-18 is raised in EAA. IL-18 can modulate either Th1- or Th2-type responses, and modulates experimental murine EAA caused by installation of preformed immune complexes [66]. The current concept suggests that the cytokine profiles in BAL fluid of EAA are incompatible with a pure Th1 or Th2 response [67,68]. Supporting data from animal models is listed below.

Co-factors

Antigen-specific antibody and lymphocyte sensitivity occur in subjects with and without EAA, although the balance of evidence suggests that levels are higher in symptomatic subjects. EAA in subjects with no immunological sensitization is rare, therefore lymphocytes and antibody seem necessary but not sufficient for disease. Other co-factors must be involved. Various constitutional and environmental factors have been identified or postulated to affect disease outcome in EAA (Table 3). Exposure to infectious particles or microbial products including bacterial endotoxin or fungal glucans in organic dust can have immunomodulatory effects which may alter disease outcome to inhaled antigens. Viral infection can up-regulate the co-stimulatory molecules CD80 and CD86 on human alveolar macrophages [26], and evidence of marginally higher viral infection with influenza A have been found in patients with EAA [69]. In mouse models of EAA, respiratory syncytial virus [70] and Sendai virus infection enhanced and perpetuated experimental EAA; the proposed mechanism for this effect was by promoting an environment which promoted Th1-type lymphocyte proliferation [71]. Constitutional factors supporting a specific immune-sensitivity imply an association of EAA with a particular HLA haplotype. Previous studies looking for such a linkage have been unconvincing, but recently with better awareness of disease and technology, pigeon fanciers with EAA were found to have a significant increase of the alleles HLA-DRB1*1305 and HLA-DQB1*0501, and a decrease of HLA-DRB1*0802 [59]. Molecular modelling may now be able to confirm the specificity of a putative disease-specific antigen [35].

ANIMAL MODELS

The disease heterogeneity of EAA and the limited access to BAL in patients tends to drive research in animal models. There are many animal models [72], with the limitation that most require adjuvant costimulation. The most commonly studied model of EAA is the instillation of spores of *M. faeni* in mice. Initial observations supported a Th-1 type lymphocyte mediated pathogenesis. Disease is associated with overproduction of IFN-γ, and is attenuated by IL-10 [67]. IFN-γ is essential for the inflammation and granuloma formation, and C57BL/6 mice that lack IL-10 have a more severe granulomatous inflammatory response [73]. Cultured antigen-sensitized Th1-type but not Th2-type cell lines can adoptively transfer experimental EAA. These Th1 cell lines are CD45RB negative, CD44 positive, CD25 low, and CD49d α4 integrin positive, which are characteristic of activated/memory T cells [74]. T cell activation is required for experimental EAA, and this is inhibited by the composite molecule CTLA4-Ig, an antagonist of the CD28/B7 interaction which is essential for complete T cell activation and differentiation [75].

Table 3. Some constitutional and environmental factors which may affect susceptibility to antigen and the development of EAA. This list summarizes factors mentioned in the text

Constitutional	Environmental
Cigarette smoking	Variable antigen exposure
Age (childhood)	Antigen concentration
HLA haplotype	Dusts (particle size)
Cytokines	Immunomodulation
Acute symptoms	Virus infection
Polymorphism	Bacterial LPS and fungal glucan [77]
Tachyphylaxis	
Subclinical disease progression and tissue remodelling/fibrosis	

There is a strain dependency for murine EAA, with sensitive (C57BL/6) and resistant (DBA/2) strains [76]. DBA/2 mice can become sensitized if they received IL-12 augmentation therapy at the time of antigen exposure [76]. A comparison of these two strains by microarray technology may determine the relevant cytokine (and other) gene expression involved in pathogenesis [56].

SUMMARY

EAA is a prototype parenchymal inflammatory lung disease. Because the populations at risk can be identified and the relevant antigens purified, EAA allows investigation of constitutional and environmental factors associated with the immunopathological responses in the lung to inhaled antigens. Apart from cigarette smokers, most subjects exposed to an antigenic aerosol produce antibody and sensitized lymphocytes, and symptoms are most evident in those with the strongest responses. This and the characteristic lymphocytic alveolitis suggest an immunological diathesis, with current evidence favouring a Th1-type mechanism. Antibody and lymphocytes seem necessary but not sufficient for symptoms and disease. Acute lung inflammation and systemic symptoms suggests proinflammatory cytokine activity, which may be absent or attenuated in subclinical inflammation allowing tissue-remodelling cytokines to determine whether disease becomes chronic. The amount of inflammatory or regulatory cytokines produced at each of these stages may determine the clinical outcome of antigen exposure. This amount may be determined constitutionally by polymorphism of cytokine genes, or may be determined environmentally by the effects of cigarette smoking or intercurrent infection on dendritic cell or regulatory lymphocyte function. These topics in basic cell and molecular biology can be addressed in the context of EAA and should provide insight into the protective immunological repertoire of the lung when dealing with inhaled antigens and the pathogenic repertoire contributing to a range of lung diseases.

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