Cells, Enzymes and Interstitial Lung Disease.

The Philip Ellman Lecture

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Amongst his many and varied contributions to medicine, Philip Ellman is remembered especially by clinicians for his appreciation of the overlap between various connective tissue disorders and lung disease. Many of these conditions are relatively rare and so individually perhaps less deserving of detailed study, but an understanding of the cellular mechanisms in some of these uncommon diseases can be used to build up concepts that are relevant to the pathogenesis of the whole spectrum of fibrosing lung diseases, including those due to organic and inorganic dusts, microbial agents and those of unknown cause. These studies go some way to throwing further light on the elegant clinical observations made by Ellman some thirty years ago.

We use cryptogenic fibrosing alveolitis (CFA) as a natural model of chronic inflammation in human lung, but contrast this at various points with other forms of chronic lung disease to illustrate their distinctive natural history and pathology, both in conventional terms and in the new ones described here.

This review is intended to stimulate ideas and to this end we have not hesitated to speculate ahead of the confirmed facts and include a number of our unpublished observations.

The Clinical Problem

While several chronic fibrosing lung disorders such as sarcoidosis, hypersensitivity pneumonitis and CFA have many distinctive features, they do share a number of common ones. Each has an identifiable active cellular stage, with differing histological features. Each on occasion progresses to end stage fibrosis, the frequency and rate of development of which varies greatly both between diseases and between individuals. Thus probably less than 10 per cent of patients with Stage II and Stage III sarcoidosis develop untreatable fibrosis; the majority resolve spontaneously over two to three years and some resolve radiographically with corticosteroid treatment. Little is known about factors influencing the natural history of sarcoidosis but the possible influence of genetic factors is suggested by the report that HLA B8 is more frequent (P<0.01) amongst spontaneously remitting cases compared with those who progress[1].

The natural history of chronic inflammatory lung disease, and factors influencing it, can be studied more easily when the causal agent is known. In hypersensitivity pneumonitis it appears that only the minority of those exposed develop the disease; thus less than 10 per cent were affected in one epidemiological study of Scottish farmers[2]. The frequency of lung involvement depends at least in part on the intensity of exposure and was twice as common in wetter parts of Scotland compared with drier areas. When a large number of those exposed remain healthy, the possibility of a varying immunological responsiveness in the host must be considered. Allen et al.[3] suggested that sensitisation of blood-derived lymphocytes was more closely related to the development of avian protein hypersensitivity pneumonitis than the presence of circulating precipitins, and others have now shown specifically sensitised lymphocytes from lung lavage samples from patients in whom lymphocyte sensitisation could not be demonstrated in peripheral blood samples[4]. On the other hand, Stokes et al.[5] have shown that the sub-class IgG, was significantly higher in patients with disease compared with matched healthy but exposed farmers, while IgG, was raised in farmers with and without disease compared with unexposed controls. These studies suggest that a propensity to produce IgG, may be an important predisposing factor to the development of some forms of hypersensitivity pneumonitis. Some cases continue to have recurrent acute clinical episodes, with or without radiographic shadowing, for many years, while a small number progress to irreversible fibrosis. Moreover, a few develop this very rapidly and even continue to deteriorate after withdrawal from exposure. This uncommon but important sequence suggests that perpetuating circuits of fibroblastic proliferation or collagen condensation, which are no longer dependent upon the primary agent, may occur. By contrast to both sarcoidosis and hypersensitivity pneumonitis, CFA has in general a much more serious prognosis (Fig. 1) and several recent large series have shown that about half of the patients have died within five years of their first hospital attendance (Fig. 2). However, in this condition, the rate of development is variable and some cases survive with apparently burnt out and stable fibrosis for 20 or more years[6].
In sarcoidosis and hypersensitivity pneumonitis spontaneous resolution is common but, with the exception of the cellular variant often termed desquamative interstitial pneumonia (DIP), this benign course is exceedingly uncommon in CFA[7]. In spontaneously unresolved cases of each of the three conditions, some subsequently respond to corticosteroids. In sarcoidosis and hypersensitivity pneumonitis response is frequent, provided treatment is begun before there is substantial fibrosis, and the responders are often predictable from the type of radiographic shadowing (i.e., those with widespread nodularity without linear shadows or radiographic reduction of lung volumes). In CFA this is not so[6,8], the frequency of any radiographic response is also much lower (less than 20 per cent) and at best the extent of resolution is in most instances only partial.

From these observations, the clinical problem must now be restated in terms of the sequence and relative intensity of different types of tissue changes in the lung and the three models of interstitial lung disease can be explored using new techniques in an attempt to explain the varied clinical patterns of disease.

The emphasis of our studies has been placed on the clinical questions requiring an answer. For example, in CFA, what are the factors predictive of steroid responsiveness on the one hand and resistance on the other? How do rapidly progressive cases differ from more slowly progressive ones? What factors distinguish burnt out stable scarring from aggressive and often lethal fibrosis of the lung? Is the latter dependent upon uncontrolled chronic inflammation or is it due to uncontrolled fibroblastic proliferation with continued collagen formation? Evidence comparing biopsy and autopsy material suggests that uncontrolled fibroblastic activity with progressive scarring may be an important component, because cases progressing fairly rapidly to death and examined at autopsy have relatively less active inflammation[6]. This implies that a possible difference between end stage healing and unstable aggressive fibrosis depends on factors controlling the dynamics of collagen turnover.

The Value of Lung Biopsies

Until recently, staging of disease has been limited to lung biopsies, and some information has been of clinical value. Wright et al.[8] using a semi-quantitative scoring system for inflammatory cells and the extent of fibrosis, found that samples showing more cellularity and less fibrosis were related to better radiographic improvement on corticosteroid treatment. The cellular biopsies have also been shown to indicate a markedly better prognosis in terms of survival, due especially to the influence of corticosteroids[9]. However, biopsy material has several limitations. The disease is often irregularly distributed within the lung and biopsy samples may not be representative. While the polar, cellular and fibrotic patterns may be helpful predictors of response, the majority of biopsies in many series are mixed[10]. Further, a biopsy can usually be undertaken only once during the course of the disease and changing patterns cannot be studied.

Bronchoalveolar Lavage

The study of pathogenesis has been transformed by the development of the technique for bronchoalveolar lavage[11]. This technique is simple and of only minor inconvenience to the patient[12]. Samples can be obtained that under certain conditions, especially the absence of obvious bronchial disease, reflect with reasonable accuracy acinar events[10,13,14]. Living cells can be obtained for a variety of biological measurements and the procedure can be repeated as often as is necessary.

Several studies have now shown that approximately $1 \times 10^5$ total cells are obtained per ml of lavage fluid recovered from healthy non-smokers[15,16]. The majority are alveolar macrophages; a small percentage are lymphocytes. It is now agreed between a number of centres that lymphocytes number around 10 per cent[17,18]. Neutrophils may occur in small numbers (less than 4 per cent) and eosinophils are virtually absent. Smoking has an important influence on cell counts. The total count is increased about twofold and is mainly due
to an increase in the number of macrophages, many of which contain pigmented tobacco-related inclusions; there is also a slight increase of neutrophils, so the percentage of lymphocytes tends to fall. However, these changes are minor compared with the very marked abnormalities induced by different types of chronic inflammatory disease. In particular, lymphocytes are increased in sarcoidosis and are especially raised in hypersensitivity pneumonitis[16]. By contrast, neutrophils and eosinophils are the predominant abnormal cell types in CFA[10,11,16-18]. A similar abnormality is seen in patients with asbestosis[10,19]. However, our own studies, using age-matched and appropriate smoking controls, suggest that lymphocyte counts can be modestly increased in CFA, though to a much lesser extent than in granulomatous disorders (Fig. 3) and similar findings have been reported by Davis et al.[20]. Moreover, it appears that in CFA, sub-groups can be identified which have mainly neutrophils and/or eosinophils on the one hand, and mainly lymphocytes on the other.

Studies on cell populations and supernatants of lavage fluids have explored their role in pathogenesis. Du Bois et al.[21] have shown that alveolar macrophages from patients with CFA show greater spreading, with the development of elongated processes on glass after 24 hours' culture, compared with normals or patients with sarcoidosis (Fig. 4). The appearances suggest that these cells are in an 'activated' state and many factors are known that induce such changes under experimental conditions. The role of immune complexes and lymphokines is of special interest.

![Fig. 3. The differential neutrophil and lymphocyte counts in bronchoalveolar lavage samples from 43 patients with cryptogenic fibrosing alveolitis. The broken line ---- indicates upper limit of values in 'normal' mixed smoking and non-smoking populations.](image)

Many years ago, evidence of local immune complexes in the lungs of patients with CFA was obtained using immunofluorescent methods on lung biopsy specimens[22] and later confirmed[23,24]. Further, increased numbers of pyronin positive cells, some of which contain immunoglobulin, have also been recognised for many years and their capacity to liberate antibody was recently demonstrated[17]. Increased levels of Clq binding in peripheral blood have now been found in over 50 per cent of patients with CFA[23,25], suggesting (but not proving) the presence of circulating immune complexes. More evidence for this comes from the demonstration of reduced antibody cytotoxicity[26]. In this test 51Cr labelled target cells (Chang cells) are coated with appropriate antibody and inter-reacted with peripheral blood lymphocytes. Under normal conditions these cells interact through their Fc receptors, inducing cytotoxic effects on the target cells with liberation of the radioactive label. If the Fc receptor sites are already occupied by prior in vivo attachment of circulating immune complexes, the cytotoxic capacity of these cells is reduced. Our studies have also demonstrated a correlation between cases showing a reduced antibody-dependent cytotoxicity and increased Clq binding (P<0.05). The fact that C5b and Fc receptor sites on the surface of alveolar macrophages are not increased in CFA[27], as might be expected in view of
their marked 'activation' in terms of spreading, could be explained if receptor sites on the macrophage membrane are already partially occupied by immune complexes. This suggestion is supported by Hunninghake et al. [28]. The demonstration of significant C1q binding in lavage samples provides further support for the presence of local immune complexes in CFA \([29,30]\).

Activation of macrophages may also be induced by lymphokines derived from sensitised and antigen-stimulated lymphocytes\([31]\). As yet, no primary 'causative' antigen has been identified in CFA, so experimental proof of specific lymphocyte sensitisation cannot be obtained at present. However, lymphocyte sensitisation to two other groups of antigens is known in this condition. Haslam et al. [32] showed that extracts of crude nuclei and DNA caused inhibition of leucocyte migration when added to peripheral blood white cells from patients with CFA. Kravis et al. [33] demonstrated inhibition of macrophage migration in a two-stage test using lymphocytes from CFA patients and Type I collagen. It is probably more reasonable to suppose that these events are secondary to tissue damage induced by some other primary event than that they represent a primary 'auto allergic' cause of the condition. In any case, they provide possible secondary circuits, promoting continuing macrophage 'activation' that might no longer be dependent upon primary events and which, in turn, might induce further tissue damage.

One of the many effects of macrophage 'activation' in experimental models is to increase intracellular levels of lysosomal enzyme and its secretion\([31]\). Indeed, this is one important accepted mechanism of macrophage-dependent tissue damage. Du Bois et al. [21] showed that intracellular levels of \(\beta\)-glucuronidase in glass adherent cells (mainly alveolar macrophages) were reduced rather than increased. However, when this lysosomal enzyme was measured in the supernatant lavage fluid and expressed as a percentage of intracellular levels, marked increases were found in CFA compared with normals (Fig. 5a, b). While it is recognised that such enzymes are derived from many other types of inflammatory cells in CFA, the data are compatible with the suggestion that lysosomal enzyme in supernatant fluid has been secreted, at least in part, from 'activated' alveolar macrophages. Such proteolytic enzymes are known to damage neighbouring cells and induce tissue injury.

Another enzyme of great interest in the context of fibrosing lung disease is collagenase. This is formed and secreted from macrophages\([34]\) and other phagocytic cells, and increased levels have now been identified in lavage fluids from patients with CFA\([35]\). Collagenase may play two quite distinctive roles in chronic inflammatory diseases of the lung, first in the control and modelling of collagen and second in relation to its effects on complement.

Collagenase specifically splits Type III and Type I collagen and its effectiveness in this depends on the physical and chemical state of the collagen fibres and on the amounts and effectiveness of a range of collagenase inhibitors. The balance achieved between collagenase and its specific inhibitors may be of crucial importance in determining the extent of established fibrosis in the lung. Little has been done to explore the clinical role of collagenase inhibitors. They can be measured in serum\([36]\) and, using a method of kinetic partition, preliminary studies suggest binding differences when serum from patients with CFA is compared with serum from patients with sarcoidosis or from normal subjects\([37]\).

Equal in importance to factors controlling the destruction of excess collagen are those that stimulate fibroblastic proliferation. Early studies by Heppleston and Styles\([38]\) suggested that macrophages again played an essential role. They found that silica-stimulated

![Fig. 5. Lysosomal enzyme levels (measured on \(\beta\)-glucosaminidase) (a) in glass adherent cells from CFA bronchoalveolar lavage material, and (b) in the lavage supernatants expressed as a percentage of the intra-alveolar levels for each patient[21].](image-url)
macrophages apparently secreted a soluble factor that increased proline uptake by fibroblasts in tissue culture. With various modifications and using different techniques, support for the general thesis that soluble factors derived from stimulated macrophages induce fibroblastic activity has come from several groups [31, 39, 40].

The importance of this discussion lies in the fact that fibrosis in human lung disease should no longer be regarded as immutable end-stage scarring, and the factors controlling its synthesis and degradation need to be sought and defined. The dynamics of collagen metabolism have been studied in a number of experimental models [40] and its reversibility under some circumstances has been clearly demonstrated. In particular, the potential reversibility of Type III collagen has been shown [41]. Few studies have been undertaken in series of patients with lung disease to study collagen types and their potential reversibility. However, recently Bateman et al. [40], using immunofluorescent methods and specific antibodies to Type I and Type III collagen, have shown a close correlation between rapidly progressive disease (in terms of the rate of clinical, physiological and radiographic progression) in patients with CFA, and increases in Type III collagen (Table 1). Micromethods for measuring collagen types and their turnover are now being used by Dr Laurent and his colleagues, and may enable us to identify cases in whom collagen may appear to be quite extensive by conventional histological methods but, because of its type, remains in a potentially reversible state.

Collagenase is also of interest in a context which is independent of collagenase metabolism. These enzymes split the C5 component of complement [43] and C5 fragments so produced have been shown to be strongly chemotactic for both neutrophils and eosinophils. C5 fragments have now been identified in lavage fluids in experimental models of fibrosis [44] and their presence in CFA is being sought. C5 fragments also trigger release of mediators of various types from mast cells, an action which is independent of cytophilic, surface attached antibodies. With this in mind, Haslam et al. [45] measured histamine levels in the supernatants from lavage samples and found increased amounts in CFA (Fig. 6). In line with this observation, mast cells were observed in considerable numbers in 1 μm sections from CFA, confirming the report by Kawanami et al. [46] (Fig. 7). These studies show the potential importance of histamine and other mast cell mediators in non-atopic disease and suggest a mechanism of induction. Preliminary studies to screen for other factors that induce vascular permeability have demonstrated their presence, and their nature is now being identified (Morley et al., unpublished observations). These studies are important because they provide further understanding of the nature of the inflammatory processes in the lung and also suggest the type of anti-inflammatory drugs that might be used to control them.

If eosinophilic chemotactic factor can also be detected from such mast cell stimulation, this might partially explain the increase in eosinophils found in some cases of CFA, an accumulation which could thus be independent

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Table 1. The correlation between identifiable Type III collagen in lung biopsies from patients with CFA and clinical assessment of activity of disease, monitoring symptomatic, physiological and radiographic changes.

| Stable | Deteriorating |
|--------|---------------|
| Excess Type III | 0 | 12 |
| No Excess Type III | 10 | 2 |
| $X^2$ 18.7 | $P<0.001$ |

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Fig. 6. Histamine levels in lavage fluids from patients with various fibrosing disorders [45].

Fig. 7. A mast cell in an alveolar wall from a patient with CFA. 1 μm section stained with toluidine blue.
of IgE and cytophilic IgG mediated reaction. In addition to mast cell mediated reactions, low molecular weight chemotactic factors for neutrophils and eosinophils have now been identified, which are released from 'activated' macrophages\[47,48\] and provide a pathway for granulocyte attraction which is dependent on macrophage activation, but independent of collagenase.

There is also important evidence suggesting that a variety of proteases secreted from both macrophages and granulocytes stimulates the alternative pathway of complement activation\[49\]. Stimulation of such a pathway could result in the recruitment of more inflammatory cells, and would in turn re-activate the macrophage. Furthermore, macrophages appear to be able to secrete both C3 and factor B so that such an amplification pathway of inflammation becomes independent of antigen and serum, the macrophage itself containing all the factors necessary to re-stimulate the circuit of its own activation.

Thus, using only a very simple and grossly incomplete scheme, a number of potentially important amplification circuits can be identified, of which there is now direct evidence in chronic lung disease in man, and which are capable of perpetuating inflammation in the absence of a continuing primary agent or antigen (Fig. 8). These circuits may be of great importance in those lung diseases clinically recognised as running a progressive course, and especially in those in which antigen can no longer be, or has never been demonstrated.

**Clinical Applications**

Lastly, we return to the clinical application of this information to the treatment of our patients. Can such studies be used to identify steroid responsiveness and suggest modes of action of these drugs? The clinical data are in some ways ahead of our fundamental understanding of the action of corticosteroids. Our own follow-up studies using either overall clinical (subjective change in dyspnoea) or objective physiological changes in forced vital capacity show a correlation between a good response to corticosteroids and the percentage of lymphocytes in lung lavage fluids in CFA (Table 2). Although this type of cell is less dramatically increased than are neutrophils and eosinophils, it appears to be a more valuable indicator of steroid responsiveness. There is also a correlation

**Table 2.** The forced vital capacity (FVC) improvement on corticosteroids and differential cell counts in lung lavage\[50\]. (No. = 29).

| Response (>10%>1 yr) | No Response | P (Fisher's Exact) |
|----------------------|-------------|--------------------|
| (n = 9)              | (n = 20)    |
| Lymphocytes>11%      | 7           | 1                  | <0.001 |
| Eosinophils>3%       | 2           | 13                 | <0.04  |
| Neutrophils>4%       | 5           | 18                 | <0.05  |
| + Neutrophils>4%     | 5           | 18                 | <0.05  |

**Fig. 8.** *A simplified scheme summarising some modes of cellular interaction, most of which have been identified in CFA.*
between those with greater than normal numbers of lymphocytes and those with increased Clq binding measured either in the blood or lavage fluid (Table 3).

Table 3. The correlation between inflammatory cells and Clq binding in lung lavage fluid and blood[45].

| Lavage Cell | Blood rs | Blood Signif. | Lavage rs | Lavage Signif. |
|-------------|----------|---------------|-----------|---------------|
| Neutrophils | 0.12     | NS            | -0.20     | NS            |
| Eosinophils | -0.13    | NS            | -0.08     | NS            |
| Lymphocytes | 0.31     | \(P<0.025\)  | 0.28      | \(P<0.05\)   |

\(rs = \) Spearman Rank Correlation Coefficient

Increased lymphocytes in the lavage fluid also relate to increased amounts of Type III collagen in the lung biopsy (Table 4). Further support for the suggestion that the percentage of lymphocytes in lavage fluid is important in steroid responsiveness is the observation that lymphocytes are also found in increased numbers in the wash fluids in sarcoidosis and extrinsic allergic alveolitis, both conditions responding much more readily and completely than CFA to corticosteroids. Thus, a number of new markers for corticosteroid responsiveness appears to be emerging.

Conversely, increased numbers of neutrophils and eosinophils in the pre-treatment lavage fluid relate to absence of response to steroids in CFA (see Table 2). Persistent deterioration over one year post-lavage and despite treatment is also significantly associated with increased numbers of eosinophils[50].

Preliminary studies of lung wash samples from patients with asbestosis have demonstrated mainly neutrophils and eosinophils[19, and P. L. Haslam, personal communication]. This is of clinical interest because, although not yet substantiated by clinical trials, most clinicians agree that the majority of these cases are steroid resistant.

These clinical observations stimulate speculation about the mechanism of action of corticosteroids in our patients. Werb et al.[51] demonstrated the action of dexamethasone on glucocorticoid receptors on the cell membrane of macrophages in culture and their effect on the production of elastase, collagenase and plasminogen activator from these cells. The test system is available to assess whether corticosteroid-resistant cases have reduced...
numbers of glucocorticoid binding sites. Corticosteroids are known to inhibit lymphokine mediated responses, which would be in line with their effectiveness in cases with relatively more lymphocytes. Steroids are also known to have relatively little effect on the properties of tissue granulocytes, such as their chemotactic responsiveness, or upon some mast cell mediated reactions. Much more needs to be known about the specific and non-specific effects of corticosteroids in concentrations likely to be present in tissues before the full explanation of their effects (and their lack of effects) in inflammation can be explained.

While these correlations between biological events and corticosteroid responsiveness stimulate exciting new ideas, it must be recognised that the relationships which have been discussed are no more than trends and many individual patients will prove to be exceptions to the generalisation set out here. These correlations are incomplete, due no doubt to our inadequate knowledge and in part to the fact (which should never be overlooked) that the study of blood and lung lavage still provides only indirect access to the third compartment, the lung tissue itself. Fascinating though the facts revealed by lung lavage are, they cannot be expected to provide exactly the same information as would come from analysis of the lung tissue.

From these many observations a general hypothesis emerges which may now be further tested. In its simplest terms it is stated thus. If there is no increase in any type of inflammatory cell, either a relatively normal part of the lung has been sampled or the disease is burnt out and stable. If excessive numbers of lymphocytes are found, and irrespective of the clinical syndrome, the condition is likely to be spontaneously reversible or reversible with corticosteroids. On the other hand, if an excess of neutrophils and/or eosinophils is found, these cases (again irrespective of the clinical syndrome) are likely to progress relentlessly and are often unresponsive to corticosteroids. Clearly, the latter two groups should overlap, and this has been found to be the case. Whether immunosuppressant agents have a wider range of activity than corticosteroids has yet to be studied. The importance of this hypothesis lies in the idea that differential cell counts in lung lavage may be of more clinical value in assessing activity of disease and its potential responsiveness to various drugs than in simply indicating the diagnostic category to which the individual may belong. The fun of a hypothesis of this sort is that it may be tested, and the risk is that it may be wrong. But either way we shall have learnt more.

The focus of attention should now be directed towards identifying other agents that may interrupt circuits which are unaffected by corticosteroids.

We now have tools that are useful in the direct monitoring of activity and likely responsiveness to certain drugs, and that give us the opportunity of understanding more about pathogenesis and the mechanisms of action of the drugs we use. With this knowledge it should be possible to apply appropriate therapy on a scientific basis in the right type of case at the right (and reversible) stage of disease. These principles should be applicable not only to a wide range of chronic inflammatory conditions of the lung but, by analogy, should point the way to better medication in many chronic inflammatory diseases of other systems, such as connective tissue disorders which also affect the lungs.

The 1980 Philip Ellman Lecture was delivered by Margaret Turner-Warwick, but much of the work described was undertaken by other members of the team.

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Journal of the Royal College of Physicians of London Vol. 15 No. 1 January 1981

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**SIMPLE LOW COST MEASUREMENT OF METABOLIC EFFECT OF LEAD INTOXICATION**

The Aviv ZPP meter is a single channel front surface fluorometer dedicated to the analysis of Zinc Protoporphyrin (ZPP) in whole blood. The detection of elevated levels of ZPP has been shown to be an invaluable method for screening children and industrial workers for chronic lead intoxication.

**FEATURES**

- Automatic sample insertion
- Insensitive to contamination of sample
- Measures ZPP not lead in blood or environment
- No volume measurement required
- Digital readout in approximately 5 seconds
- Only finger puncture sample required
- Simple and economical to operate

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