Immunological Investigations of Coalworkers' Disease

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The pathogenesis of complicated pneumoconiosis in coal workers is still obscure despite the large amount of data collected from epidemiological, pathological and other studies carried out in the United Kingdom during the last twenty years. Earlier concepts of pathogenesis implicating silica, and then tuberculosis, have been challenged, and alternative hypotheses invoking an immunological mechanism have been suggested on the basis of the histological features and the frequent occurrence of positive serological tests for rheumatoid factor. It was considered, therefore, that an attempt should be made to define the possible significance of rheumatoid factor titre in relation to the radiological category of the disease, and, by immunofluorescence techniques, to determine the site of production and the distribution of rheumatoid factor in relation to the lesions. Only the preliminary results are reported.

Fletcher and Gough (1950) defined complicated pneumoconiosis as 'the combination of simple pneumoconiosis and progressive massive fibrosis', 'simple pneumoconiosis' being used to describe the widespread minute opacities that occur in the lungs of men exposed to heavy clouds of coal dust, and 'progressive massive fibrosis' (PMF) to describe the localised, more homogeneous opacities of larger size.

INCIDENCE OF COMPLICATED PNEUMOCONIOSIS

The incidence of complicated pneumoconiosis among working miners is about 5,000 or 1 per cent of all working miners in Britain, with an incidence of about 4 per cent in South Wales. The number of pensioned miners with the disease has been estimated to be at least twice as many as those working (Gilson, 1964). Cochrane (1961a, 1961b) has shown that complicated pneumoconiosis is a cause of appreciable disability and loss of life expectancy.

THE ASSOCIATION OF RHEUMATOID ARTHRITIS AND COMPLICATED PNEUMOCONIOSIS

Caplan (1953) originally observed a correlation between rheumatoid arthritis and the development of complicated pneumoconiosis. As seen radiologically,

the larger opacities in the lung tended to be more rounded, appeared in batches and there was less evidence of simple pneumoconiosis than in ordinary progressive massive fibrosis. The association with rheumatoid arthritis was proved conclusively by the epidemiological field studies of Miall *et al.* (1953).

The pathological features of this condition were described by Gough *et al.* (1955). They stressed the difference in the macroscopic and microscopic appearances of these lesions from those seen in orthodox pneumoconiosis, although the distribution of the lesions was the same in both conditions. Where masses were present they were seen to consist of confluent nodules. Cavitation of the massive lesions was frequent, giving the appearance of tuberculosis. Another feature was the concentric appearance of the nodules, with alternating dark and pale yellow, or grey zones: liquefaction was common in the paler areas. As the lesions progress the dust is left in the necrotic areas, giving a concentric tidal appearance.

Histologically, the main differentiating feature of the nodules was the presence of a peripheral zone of active inflammation. This zone consisted of necrotic collagen infiltrating polymorphonuclear leucocytes and disintegrating macrophages which contained the dust that was then deposited and which accounted for the concentric rings that indicate previous periods of activity. Gough (1959) commented that diagnosis might depend on the regularity of these rings when the acute phase had 'burnt out' and in some cases it was extremely difficult to differentiate the disease from old tuberculosis.

Increased collections of lymphoid cells were observed around the periphery of the lesions, and lymphocytes and plasma cells were frequently seen in the obliterated lumina of the blood vessels. Originally it was thought that these findings were not sufficiently different from those in orthodox cases to be distinctive. However, Gough (1960, 1965) now considers them to be significant and part of the diagnostic criteria.

SEROLOGICAL INVESTIGATIONS

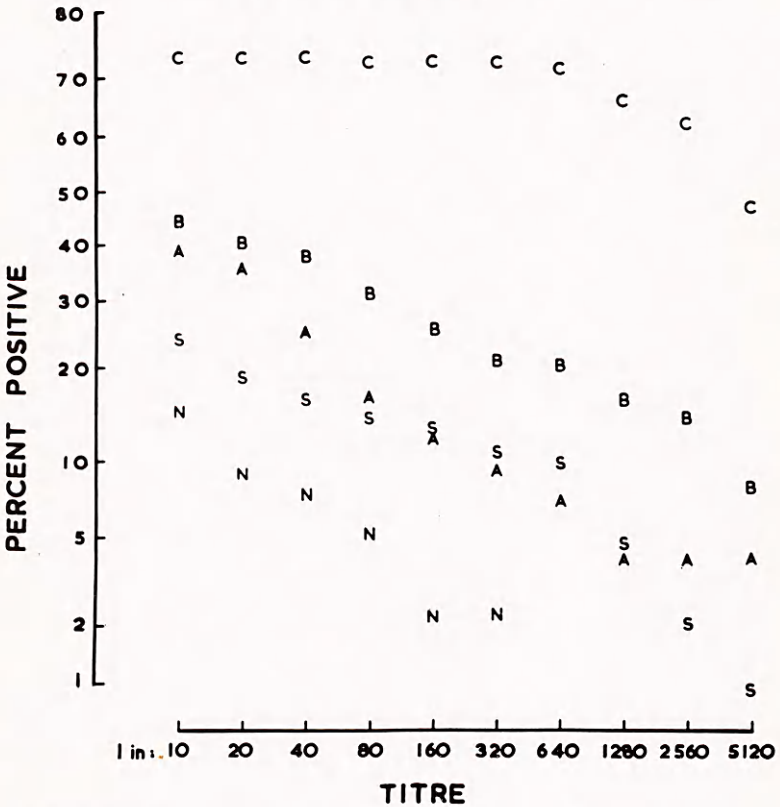
From the evidence available it appears that the spectrum of rheumatoid pneumoconiosis may be wider than was originally realised. In 1962, the radiological diagnostic criteria were extended to include features previously placed in the category of progressive massive fibrosis. In such cases, satisfying the extended criteria, positive serological tests for rheumatoid factor were common even in the absence of overt rheumatoid arthritis (Caplan *et al.*, 1962).

In collaboration with Professor B. Pernis, therefore, it was decided to correlate rheumatoid factor serology with clinical and radiological status in the following groups:

1. Control group of non-miners (N)
2. Miners with simple pneumoconiosis (S)
3. Miners with early PMF lesion ('A' shadows) (A)
4. Miners with moderate and marked PMF ('B' and 'C' shadows) (B)
5. Cases of Caplan's Syndrome (C)

Sera from 80–100 men in each group were titrated against heat-aggregated human and rabbit gamma-globulin, adsorbed on tanned sheep cells. The results are shown in Table 1.

TABLE 1. Aggregated Human Gamma-Globulin Titre Related to X-ray Category of Pneumoconiosis



C = Caplan B = Cat. B and C A = Cat. A S = Cat. 2 and 3 N = Non-miners

It is notable that the frequency of positive results and the range of titres corresponds closely with the radiological order of progression of pneumoconiosis. On the whole, there were fewer positive tests with rabbit gamma-

globulin as reactant, and titres were lower than with human gamma-globulin. In both test systems, however, there was a clear distribution of the highest titres in the cases of Caplan's Syndrome. There is also an apparent anomaly in the Caplan cases, where 26 per cent were negative at all titres with human reactant and 43 per cent with rabbit reactant, while in most of the remainder, tests were positive at titres of 1/640 or above in both systems.

IMMUNOHISTOLOGICAL INVESTIGATIONS

In a preliminary study of quick-frozen lung tissue from cases of Caplan's Syndrome, Pernis *et al.* (1965) demonstrated deposits of γ G-globulin and minor amounts of γ M-globulin at the periphery of lung nodules. With the exception of very small and presumably early nodules, immunoglobulins were not detected in the central zone. Rheumatoid factor could not be detected in the lesions but the immunohistological picture of the Caplan's nodule was considered to be very similar to that of the subcutaneous nodule in rheumatoid arthritis. Owing to unavoidable delays in processing there was some doubt as to whether the specimens used in this study were suitable for the detection of rheumatoid factor. Furthermore, histological examination revealed only one case which satisfied all the criteria for active rheumatoid pneumoconiosis as described by Gough *et al.* (1955). The majority fitted the pattern of 'burnt out' lesions as described by Gough (1959, 1965). In three cases there were no specific features to distinguish these lesions from the general variations of complicated pneumoconiosis. It was decided, therefore, to repeat the investigation on a larger scale in a wider selection of material, and to confine the examination to the immunohistological localisation of rheumatoid factor.

Fresh tissue was obtained from unselected cases from one locality and included specimens from all categories of coal workers' pneumoconiosis. Additional cases were also obtained from Sully and Llandough Hospitals. Both lungs were available in the majority of cases. One lung was inflated with formol acetate solution, and used for the preparation of Gough–Wentworth (1960) whole lung sections. Sections stained with haematoxylin and eosin were prepared from both lungs and used for the histological classification and for selecting suitable blocks for immunological study. These blocks, together with peribronchial and abdominal lymph glands when available, were processed by the cold alcohol-fixation paraffin embedding technique of Sainte-Marie (1962), which facilitated cutting of sections and provided better histological detail than comparable quick-frozen sections.

Fluorescein-labelled aggregated human γ-globulin (Kabi Pharmaceuticals Ltd, London) was used to detect rheumatoid factor according to the method

of McCormick (1963). A fluorescein-labelled anti-IgM antisera (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) was also used to map the distribution of IgM to confirm the specificity of the fluorescent aggregate reactions.

DISTRIBUTION OF RHEUMATOID FACTOR

Lung

By strict immunofluorescent criteria, specific staining for rheumatoid factor was observed in the cytoplasm and, occasionally, the nucleus of immature and mature plasma cells arranged around and in the walls of blood vessels, or lying in rows and small irregular aggregates among dense connective tissue at the periphery of nodules. Rheumatoid factor was also seen in well-defined lymphoid follicles, some of which showed germinal centre formation. Occasionally, spindle-shaped cells resembling fibroblasts contained rheumatoid factor; alveolar and perivascular macrophages tended to stain non-specifically. Extracellular IgM was found in areas of necrosis as streaks in the peripheral zone of fibrotic nodules and, occasionally, in the wall of vessels showing severe endoarteritis. In some cases, extracellular IgM could be identified as rheumatoid factor by its reaction with fluorescent aggregated human γ-globulin.

Lymph Nodes

Rheumatoid factor was present in variable numbers of mature and immature plasma cells and, occasionally, in germinal centres. In some nodes there were striking aggregates of brightly stained plasma cells in relation to macrophages containing dust in a fine, speckled distribution. In other cases, where there was distortion of lymph-node architecture by massive dust deposits and replacement of fibrosis, some subcapsular regeneration of lymphoid tissue was observed and rheumatoid factor was present in the subcapsular follicles and plasma cells. In most sections the distribution of rheumatoid factor reactivity corresponded closely with the pattern of staining produced by the anti-IgM antiserum.

Although the concentration of rheumatoid factor in the lung and lymph nodes varied from striking to minimal, the pattern of distribution was similar throughout the series of cases.

Immunohistological Correlation

The results are summarised in Table 2. As whole lung sections were not always available, it was decided to classify the cases on histological criteria alone.

Each case was allocated to the appropriate histological category as indicated below without prior knowledge of the immunofluorescence results.

Caplan's Syndrome Rheumatoid Pneumoconiosis

As was expected, rheumatoid factor was observed in those cases that had the histological features of rheumatoid pneumoconiosis as described by Gough *et al.* (1955). Evidence of activity was shown in 7 cases, the other 3 were 'burnt out' (Gough, 1959, 1965). Of the 2 cases that were negative, 1 had no definite

TABLE 2. Analysis of 88 Cases

Histological diagnosis	No. of cases	Rheumatoid factor present
Caplan's syndrome	10	8
PMF with vasculitis	15	10
Non-specific PMF	16	3
Simple pneumoconiosis	35	7
Silicosis	6	—
Tuberculosis	6	—
Totals	88	28

lesions in the material that was selected for immunofluorescence examination; there was no reason for the failure to obtain a positive result in the other.

PMF with Vasculitis

The second group to have a high rate of positivity were those cases of PMF with marked vasculitis in which plasma cell infiltration of the vessel walls extended into the obliterated lumina. These lesions were surrounded by numerous discrete foci of plasma cells and lymphocytes. It was not possible to differentiate on histological grounds between the positive and negative cases. Areas of necrosis were common but this was also observed in the non-specific group.

Non-specific PMF

Cases of PMF that showed no features suggestive of a definite aetiology were placed in this category. Histologically, the appearances varied from an irregular deposition of collagen fibres and coal dust to dense hyaline tissue surrounding necrotic foci. Specimens with positive staining for rheumatoid factor could not be differentiated from the others in this group. However, two of the lesions occurred in lungs in which there was definite honeycombing, a

lesion associated with rheumatoid factor (Tomasi *et al.*, 1962). In addition, one of the positive cases with simple pneumoconiosis also had honeycombing. These were the only cases of honeycomb lung in the series and all were positive for rheumatoid factor.

Simple Pneumoconiosis

Included in this category were cases in which there was fibrotic dust nodulation of less than 2 cm in diameter. Apart from the case of honeycomb lung, 6 other cases were positive among the simple group, 2 showing marked vascular change in the nodules associated with central necrosis and peri-nodular foci of lymphocytes and plasma cells. Of the remaining 4 cases, 2 showed lesions suggestive of rheumatoid foci in the peribronchial glands.

Silicosis

Six cases showed histological features that were thought to be consistent with silicosis or mixed dust lesions in which silica played a prominent part. In none of them was evidence of rheumatoid factor found. The total dust and free quartz content of 40 of the lungs in the series has been estimated, including 5 of the 6 cases thought to be silicotic. Of those 5 cases, 2 were found to have a quartz percentage of the total dust consistent with silicosis (15 per cent or above). The other 3 were well below this figure. In addition, 3 cases had a quartz percentage consistent with silicosis, but were not diagnosed histologically.

Tuberculosis

Rheumatoid factor was not detected in these cases with histological evidence of tuberculosis. One case proved positive for tuberculosis on guinea-pig inoculation and culture, two were positive on culture only. The remaining three were negative and showed no histological evidence of active tuberculosis. A similar search for tuberculosis was made in 49 cases in the series without histological evidence of tuberculosis. Culture proved positive in one case of PMF with marked vasculitis which stained positively for rheumatoid factor.

CONCLUSIONS

The relatively high incidence of positive rheumatoid factor tests in Caplan's syndrome and PMF with vasculitis and the serological anomaly found in the Caplan's group might be explained by radiological overlap and possible interchange between the two groups. This tentative conclusion is apparently supported by the results of the immunohistological correlation. It may be

significant that rheumatoid factor, and therefore immunological overlap with Caplan's Syndrome, occurs mainly in those cases of PMF with marked vasculitis and plasma cell infiltration. It is possible that this category of PMF may represent an intermediate group that may not be differentiated from massive Caplan's Syndrome or non-specific PMF on radiological assessment alone. Whether or not this intermediate group is a distinct entity may be determined at a later date by full correlation of clinical and radiological data with the results of this present investigation.

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