Immunoglobulins and complement in pleural effusions associated with bronchogenic carcinoma

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SYNOPSIS Levels of IgG, IgA, IgM, the total haemolytic complement (CH_{50}), and the individual components C1q, C3, C4, C6, and C7 were measured in 29 pleural effusions. Of these, 18 were associated with carcinoma of the bronchus and 11 were non-malignant effusions including empyemas.

The level of IgG was significantly lower in the malignant group when compared with non-malignant effusions. The usefulness of measurements of IgG with respect to malignant effusions associated with carcinoma of the bronchus requires an expanded study to show whether it has any real diagnostic value. There were no significant differences in other immunoglobulins, the CH_{50} , and individual complement components between the two groups.

The identification of total haemolytic activity in the majority of effusions in both groups indicates that all nine components of the classical pathway of complement, including macromolecules such as C1, can be present in pleural fluids.

There have been relatively few reports on measurements of immunoglobulins and complement in pleural fluid. Hunder *et al* (1972) found that the total haemolytic complement (CH_{50}), C4, and C3 concentrations were lower in the pleural fluids from patients with rheumatoid arthritis and systemic lupus erythematosus than in fluids from patients with malignant and other diseases. Hirsch *et al* (1971) reported the presence of immunoglobulins IgG, IgA, and IgM in nine pleural fluids in amounts comparable to those concentrations present in the serum.

Since there have been a number of reports which suggest that cancer patients have circulating antigen-antibody complexes which in turn may lead to activation of complement, we have measured immunoglobulins and individual complement components in 18 malignant pleural fluids and compared these with 11 non-malignant effusions, including empyemas.

Material and methods

PLEURAL EFFUSIONS

Samples were centrifuged at 1500 *g* to remove parti-

culate matter, divided into portions, and kept at -80°C . The effusions were processed and stored on the same day as the pleural aspiration and were thawed only once before each assay.

TOTAL PROTEIN

This was measured by a standard AutoAnalyzer (Technicon Instruments Corporation Ltd, Basingstoke) method (N14b) which uses the biuret reaction. The between-batch precision (± 1 SD) was 1.0 g/l.

IgG, IgA, AND IgM

These were measured by an automated nephelometric procedure on an AutoAnalyzer II (method no. 12). The method incorporates 4% polyethylene glycol in the antiserum diluent. Samples were pre-diluted manually (1:50 for IgA and IgM, and 1:200 for IgG) rather than on the AutoAnalyzer. The between-batch precision (± 1 SD) in the range up to 300 IU/ml was 8.0 IU/ml for IgG, 12.6 IU/ml for IgA, and 7.0 IU/ml for IgM.

COMPLEMENT

The haemolytic complement (CH_{50}) was measured by the method of Mayer (1961). Levels of complement components C1q and C3 were measured by single radial diffusion using monospecific antisera

raised in the rabbit. Levels of C4 and C6 were measured using C4-deficient guinea-pig and C6-deficient rabbit sera respectively (Lachmann *et al*, 1973), and C7 was measured by the reactive lysis method (Thompson and Lachmann, 1970). Immunoelectrophoresis was performed as described (Scheidegger, 1955) using a rabbit antiserum to human plasma proteins purchased from Wellcome Reagents Ltd.

Results

When the electrophoretic pattern of malignant and non-malignant pleural effusion, including empyemas, was compared to the pattern of normal serum there were no major differences in the number of positions of the principal protein bands.

The amount of Ig G per milligram total protein in the malignant effusions was significantly ($P < 0.01$) lower than the IgG content of the nonmalignant group (fig 1). The differences in IgA and IgM per milligram of total protein and in total protein concentration itself between the two groups were not significant.

Total haemolytic complement was measurable in the majority of pleural effusions but there was no significant difference in levels between the malignant and nonmalignant fluids (fig 2). The individual components C1q, C4, C3, C6, and C7 were also detectable in the majority of effusions but no significant differences were found between the two groups (fig 2).

Discussion

In the report by Hirsch *et al* (1971), three out of the nine pleural effusions were from patients with

Fig 1 Immunoglobulins and total protein in malignant and nonmalignant pleural effusions. Malignant effusions (●), empyema (○), tuberculosis (■), pneumonia (□), pulmonary infarction (▲).

Fig 2 Total haemolytic complement (CH_{50}) and the individual complement components C1q, C4, C3, C6, and C7 in malignant and nonmalignant pleural effusions. Malignant effusions (●), empyema (○), tuberculosis (■), pneumonia (□), pulmonary infarction (▲).

carcinoma of the lung, but in this small series the levels of IgG, IgA, and IgM were not markedly depressed when compared with nine nonmalignant fluids. Our findings show that the amount of IgG per milligram of total protein in malignant effusions was significantly lower than the IgG content of the nonmalignant fluids, but there was considerable overlap between the two groups (fig 1). This observation may, however, have relevance when very low IgG concentrations are present although further studies are necessary to evaluate the real diagnostic value of these findings. Immunoelectrophoresis alone does not appear to have diagnostic value as the patterns were similar with each disease state.

The reasons for the lower concentration of IgG in the malignant group is unknown. One explanation is that IgG is an inadvertent substrate for plasmin as a result of local fibrin formation and activation of the fibrinolytic pathway. Disseminated intravascular coagulation (DIC) in association with disseminated carcinoma is well recognized. Although DIC was not a complication in any of the patients studied here it is possible that there was low-grade conversion of plasminogen by a plasminogen activator in the pleural space of the patients with carcinoma. Levels of fibrin degradation products were higher in pleural fluids from patients with carcinoma than in nonmalignant fluids (J. D. Cash, personal communication).

Although we did not have the opportunity to measure plasma concentrations of immunoglobulins at the time of pleural aspirations, it is unlikely that our findings merely reflect low circulating IgG levels. In a report by Hughes (1971) IgG levels in cancer patients, including those with bronchogenic carcinoma, were significantly increased when compared with those in matched controls.

Measurements of the CH_{50} and individual components in body fluids other than plasma have

been of value diagnostically. The complement levels in joint effusions in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) have been found to be lower than in 'non-rheumatoid' arthritis fluids from patients with osteoarthritis or gout (Pekin and Zvaifler, 1964; Franco and Schur, 1971). Similarly, the CH₅₀, C1q, C3, and C4 were lower in pleural fluids secondary to SLE and RA than in malignant pleural effusions (Hunder *et al*, 1972). These findings are thought to be a consequence of complement activation by antigen-antibody complexes. Since there is evidence in both man and experimental animals for the presence of immune complexes in cancer (Baldwin *et al*, 1974), in this study we have measured complement in malignant effusions and have included the terminal components C6 and C7. We have compared our malignant group with exudates associated with pneumonia, tuberculosis, and pulmonary infarction and with empyemas. Although we found no significant difference between our two groups in terms of complement measurements (fig 2), the observation that many of these components were present adds to knowledge of the composition of pleural fluids.

The mechanism by which fluid accumulates within the pleural cavity is incompletely understood. Leckie and Tothill (1965) found that radiolabelled albumin can diffuse freely out of the pleural space, and it seems likely that most proteins in pleural effusions derive from the circulation as a result of the inflammatory process rather than from local production. It is reasonable to suppose that macromolecules, such as C1 (molecular size 900 000), can diffuse into the pleura. The majority of fluids from the malignant and nonmalignant groups had total complement activity (the CH₅₀) which requires C1 and the other components of the 'classical pathway'.

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