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ASSAY OF CIRCULATING IMMUNOGLOBULINS IN PATIENTS WITH FIBROSING ALVEOLITIS

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SUMMARY

One or more classes of immunoglobulins were increased above normal in twenty-one of thirty-two patients with fibrosing alveolitis. One class of immunoglobulins only was raised in fourteen sera: IgG in eight, IgA in three and IgM in three. More than one type of immunoglobulin was increased in eight instances. In five sera the increase of IgA was sufficiently great to cause an early 'hump' on the electrophoretic strip.

The presence of a range of non-organ specific autoantibodies correlated with increased immunoglobulins and their absence with normal immunoglobulin levels in twenty-six of our thirty-two patients. In this study the presence of individual autoantibodies could not be related to individual types of immunoglobulins, owing to the multiplicity of immunoglobulins and autoantibodies in individual sera.

INTRODUCTION

Abnormally high total serum globulin values have been reported in 40% of patients with fibrosing alveolitis (Turner-Warwick & Doniach, 1965) and a range of one or more non-organ specific circulating autoantibodies have been found in over 50% of patients with this condition (Turner-Warwick & Doniach, 1965; Mackay & Ritchie, 1965).

In the sera of patients with auto-allergic disorders characterized by multiple non-organ specific autoantibodies, the increase in γ-globulins is often due to one class of immunoglobulin only (Waldenström, 1962). It has been shown that IgG is frequently increased in patients with systemic lupus erythematosus and IgM in those with primary biliary cirrhosis (Hobbs, 1966a). These findings contrast with healthy reactions to bacterial infections where a proportional increase in all immunoglobulin components is characteristic (Hobbs, 1966b).

We report here the results of quantitative assay of circulating immunoglobulins in

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thirty-two patients with fibrosing alveolitis whose sera have been tested for a range of autoantibodies.

PATIENTS

Thirty-two patients were studied in whom the diagnosis of fibrosing alveolitis was made on clinical, radiographic and physiological grounds. The presenting symptoms were increasing breathlessness often accompanied by an unproductive cough. On examination finger clubbing was present in the majority and widespread fine crepitations were heard in all except two of the patients.

Radiographically there was bilateral abnormal shadowing ranging from a patchy shadowing often most marked in the lower zones, to widespread fine or coarse mottling. In a few cases small cystic spaces could be found within areas of dense shadowing (a detailed analysis of the radiographic appearances correlated with the presence of autoantibodies and the clinical response to corticosteroids is in progress).

Physiological studies showed a restrictive ventilatory defect with impairment of gas transfer but without evidence of airways obstruction.There is now general agreement with Scadding (1960) that when the findings are characteristic using these methods of assessment, it may be predicted that the histological appearances will conform to the general description of fibrosing alveolitis and verification of this by lung biopsy is often not clinically justifiable.

There were eleven women aged between 29 and 82 years, and twenty-one men with an age range of 37–77 years. At the time of immunological study pulmonary symptoms had been present from between 6 months and 5 years in twenty-nine of our thirty-two patients, less than 6 months in two (6 and 8 weeks) and for more than 5 years in one (14 years). In all patients the dominant clinical problem was their lung disease but co-existing disorders were found in seven; three patients had polyarthralgia although none fulfilled the criteria of the American Rheumatism Association (1959) to classify them as rheumatoid arthritis. One had ulcerative colitis, one Sjögren's syndrome and two had asthma beginning in adult life.

ASSAY OF IMMUNOGLOBULINS

Immunoglobulins were measured by a modified Mancini method (Hobbs and Maatela, to be published) using radial immunodiffusion in agar containing our own monospecific antisera to IgG, IgA and IgM globulin: this method has a reproducibility of $\pm 10\%$.

The working reference standard serum was stored in aliquots at $-20°C$, these were thawed rapidly only once, used and discarded. They were calibrated at 3 monthly intervals against freshly prepared solutions of IgG, IgA and IgM globulins. As a further check on the quantitative immunoglobulin results, the total γ-globulin was estimated after serum electrophoresis and staining on cellulose acetate, and expressed as a percentage of the total dye uptake. From the total protein determined by biuret and specific gravity methods, the γ-globulin was calculated as g/100 ml. With the method used (Hobbs, 1965) IgG and IgA globulins can be assessed within $\pm 0{\cdot}2$ g/100 ml. High levels of IgM globulin are underestimated by biuret but can be measured using the specific gravity method. If the total γ-globulin of the serum did not agree within 0·2 g/100 ml with the sum of the immunoglobulins measured by the Mancini method, all would be re-checked. In fact, good agreement between the two methods was found with all the sera in this study and re-checking was not necessary.

MEASUREMENT OF AUTOANTIBODIES

Rheumatoid factor (RF) was measured using the latex F II test (Singer & Plotz, 1956) and the differential agglutination test (Rose *et al.*, 1948). Anti-nuclear factors (ANF) were detected by a double-layer immunofluorescent technique using rat liver as substrate, and titrated using serial dilutions of serum in the same test. Non-organ specific complement fixing antibodies (AICF) were titrated using rat liver and kidney as substrate. Thyroid cytoplasmic and thyroglobulin antibodies were detected by immunofluorescence. Positive sera were further tested by complement fixation to determine the titre of microsomal antibodies and by formalized tanned red cells to determine the titre of thyroglobulin antibodies (see review by Roitt & Doniach, 1958). Antibodies to gastric parietal cells were detected in a double layer immunofluorescent test using fresh snap frozen human stomach (Taylor *et al.*, 1962).

RESULTS

Serum levels in excess of the normal range of one or more immunoglobulin were found in twenty-two out of thirty-two sera from patients with fibrosing alveolitis (Fig. 1). One class

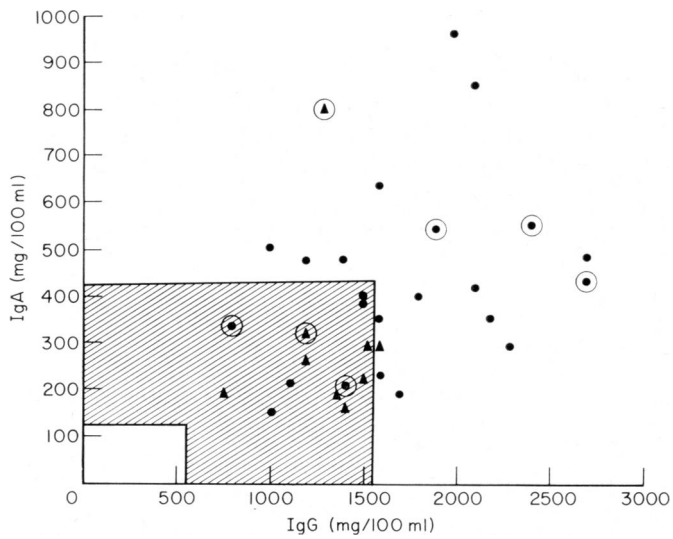

FIG. 1. Immunoglobulin levels in sera from thirty-two patients with fibrosing alveolitis. ●, with antibodies; ▲, without antibodies; ○, increased IgM; cross-hatched area, normal range (±2 S.D.).

of immunoglobulin only was increased in fourteen sera: IgG in eight; IgA in three and IgM in three. Greater than normal amounts of more than one immunoglobulin were found in eight sera, but in three of these the major increase was confined to a single class (IgG in two and IgA in one). In five sera IgA was increased sufficiently to be seen on the electrophoretic strip as an early hump lying between the β and γ bands (Fig. 2). A proportionate increase in two components was found in four sera, and in three components in one serum from a patient with known severe super-added infection.

A range of non-organ specific autoantibodies, ANF, AICF and RF were found in twenty-three of thirty-two sera tested; in addition, thyroid antibodies were found in two of these sera and also in one other. These antibodies were associated with quantitative increases in

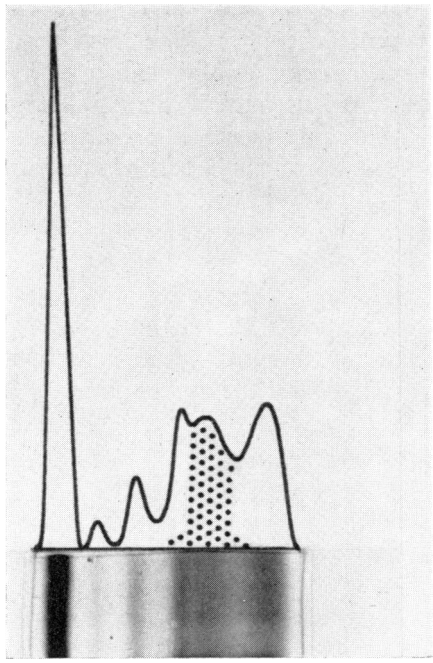

FIG. 2. Electrophoresis on cellulose acetate of fresh serum from a patient with fibrosing alveolitis. Note the hump of excess γA-globulin, seen also with Laennec's cirrhosis but otherwise rarely.

immunoglobulins in nineteen of the twenty-three sera. By contrast in nine sera without detectable circulating antibodies, the immunoglobulins were quantitatively normal in six (Table 1).

TABLE 1. Autoantibodies and assay of immunoglobulins

	Increased Ig	Normal Ig	Total
Antibodies present	19	4	23
Antibodies absent	3	6	9
Total	22	10	32

The types of autoantibodies detected in sera from our patient are shown in Table 2.

In this study it was not possible to relate individual autoantibodies with increases in individual immunoglobulins owing to the frequent finding in the same serum sample of

more than one antibody on the one hand, or more than one raised immunoglobulin level on the other.

Using mono-specific fluorescein conjugated antisera it was, however, possible to identify anti-nuclear factor localized to a single immunoglobulin component; ANF of IgG type was found in six sera and in all of these the serum IgG was raised. ANF demonstrable with

TABLE 2. Autoantibodies detected in sera from patients with fibrosing alveolitis

Patient	Sex	Duration of disease (years)	Antibodies*				Response to steroids†
			ANF	AICF	RF	Thyroid	
Immunoglobulin levels above normal							
W.G.	M	4	1/10	1/32	1/256	—	0
A.H.	M	4	—	1/16 'M'	1/64	—	0
W.G.	M	2/12	—	—	1/32	—	Not used
J.G.	M	3	—	—	1/32	—	++
E.R.	F		—	—	1/16	—	Not used
D.S.	M	1	—	—	Latex+	—	Not used
E.S.	F	4	—	1/128 'M'	—	—	Not used
H.S.	M	1	1/80	1/64	—	+	Not used
V.Mc.	M	6/12	1/10	1/16 'M'	—	—	++
P.G.	M		—	1/16	—	—	Not used
W.P.	M	4	—	1/16 'M'	—	—	0
G.W.	M		—	1/16	—	—	0
N.C.	F	9/12	1/80	—	—	—	0
W.O.	M	4	1/20	—	—	—	Not used
W.B.	M	9/12	1/20	—	—	—	Not used
B.H.	F	1	1/10	—	—	—	++
C.H.	F	3	1/10	—	—	++	+
W.A.	F	6/12	—	—	—	++	++
R.R.	F		—	—	—	—	Not used
C.W.	M	3	1/10	—	—	—	+
R.T.	M	2	—	—	—	—	Not used
S.R.	F	6/12	—	—	—	—	++
Immunoglobulin levels within normal range							
E.W.	F	5	1/100	—	1/64	—	Not used
S.Mc.	M	3	1/40	—	—	—	0
J.Mc.	F	6/12	1/10sp	—	—	—	Not used
J.D.	M	1	—	'M'	—	—	++
J.G.	M		—	—	—	—	Not used
I.H.	M	6/12	—	—	—	—	+
H.B.	M	14	—	—	—	—	0
H.K.	M	5	—	—	—	—	Not used
C.M.	M	4	—	—	—	—	Not used
I.S.	F	1	—	—	—	—	++

* —, No antibody detected; +, immunofluorescence + ve; ++, immunofluorescence and CFT + ve.
† Clinical +, improvement; ++, clinical, radiograph and/or physiological improvement; 0, no improvement.

IgM was identified in five sera, three of which had increase of serum IgM. In one instance ANF appeared to be IgM in nature in a serum containing mainly an increase of IgG. The results reported above refer to the antisera giving the strongest fluorescence but in four instances slight nuclear fluorescence was also obtained using conjugated antisera to other immunoglobulins suggesting that in these sera, anti-nuclear factor is not confined to a single immunoglobulin component.

Lung biopsies were available from two patients in this series and were studied using the direct Coon's technique with mono-specific conjugated antisera to the individual immunoglobulins. Numerous plasma cells were found in both specimens. In one fluorescence in plasma cells was mainly found using anti-IgM and in this patient IgM was the only immunoglobulin component raised above normal. In the other, fluorescent plasma cells were seen using anti-IgG which corresponded to the quantitative findings in this sera.

In this small series neither the immunoglobulin level nor the presence of autoantibodies was apparently related to favourable response to treatment with corticosteroids. Neither were there any apparent clinical or radiographic differences between patients with normal and abnormal serum immunoglobulin levels.

DISCUSSION

Increased amounts of immunoglobulin have been found in 62% of a small series of patients with fibrosing alveolitis and have been found to correlate with the presence of a range of non-organ specific autoantibodies. Hobbs (1966b) found that in organ specific auto-allergic disease increases in IgG predominantly were characteristic although sometimes predominant increase of IgM was found. Such polyclonal increases, mainly within a single immunoglobulin class may be due to the narrower antigenic stimulus in these diseases compared with that encountered against the numerous antigens of most bacterial infections, where it is usual to find parallel increases in IgG, IgA and IgM. In auto-allergic disease characterized by the presence of multiple non-organ specific antibodies serial immunoglobulin measurements have revealed increase in one component followed later by an increase in a second: for instance, in some patients with systemic lupus erythematosus or juvenile cirrhosis, anti-nuclear factor associated with IgG was found in the early stages, while later in the disease IgM serum levels increased with the concomitant development of anti-nuclear factor detected with antiserum mono-specific to IgM. The finding in four of our patients of anti-nuclear factor associated with more than one immunoglobulin suggests the same sequence of events may have occurred. The multiplicity of anti-nuclear factors of different immunoglobulin structure is likely to be a reflection of the known multiplicity of nuclear antigens (Barnett *et al.*, 1963).

The type of immunoglobulin found in disease may depend not only on the nature of the antigen but also on the time interval after antigenic stimulus (Uhr & Finkelstein, 1963). In infective hepatitis for instance an initial rise of IgM has been shown, followed after 4 weeks by a continued increase of IgG, the latter having been interpreted as reflecting the immunological response to a persisting virus infection (Lee, 1965). The sera of our patients with fibrosing alveolitis were tested after symptoms had been present for many weeks and if an analogous 'virus response' had occurred we would expect to see only the late IgG

phase. However, there is no evidence that a respiratory virus plays an aetiological role in this condition.

In twelve of our patients in whom there was an increase in only one immunoglobulin component, antibodies known to belong to other classes were sometimes found. For instance, rheumatoid factor was found in one patient with increased IgG only and in two with increases of IgA only, while in two patients with ANF associated with IgM, quantitative increases of IgG only was observed. This disparity may be accounted for by the far greater sensitivity of the techniques used for antibody detection than those used for immunoglobulin assay.

In those sera in which more than one immunoglobulin was raised above normal there was often a disproportionate increase of one component. This feature contrasts with the proportionate increase of immunoglobulins seen in most bacterial infections. In five of our cases with increased IgA, this was reflected as an early, distinct hump on the electrophoretic strip. Increases in IgA of sufficient magnitude to distort the electrophoretic strip in this way are uncommon and in the experience of one of us (J.H.) has only been seen in sera from patients with Laennec's cirrhosis and fibrosing alveolitis. Whether these appearances are sufficiently characteristic to be of any diagnostic value must remain open until studies of a large number of other respiratory diseases have been completed. These controls are essential because there is now evidence that IgA is produced by plasma cells lining the respiratory tract and secreted into the bronchi (Tomasi & Zigelbaum, 1963) and may, therefore, be a common pattern of response in a number of pulmonary diseases. Nadel *et al.* (1966) have recently reported three cases of pulmonary vascular disease apparently quite distinct from fibrosing alveolitis in which IgA only was increased.

In six sera no antibodies and normal immunoglobulin levels were found. It is unlikely that this group represents 'burnt out' cases because the duration of symptoms was no greater than groups I and II and four patients responded to corticosteroid treatment. It is possible that in these cases tissue fixation of circulating antibody had occurred. Another possible explanation of the normal findings is that there are several causes of the clinico-pathological syndrome referred to as fibrosing alveolitis, and that in some cases this may not be immunological in nature.

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