Clin. exp. Immunol. (1981) **46**, 547–556.

Radioimmunoassay profile of antiglobulins in connective tissue diseases: elevated level of IgA antiglobulin in systemic sicca syndrome

K. B. ELKON, F. CAEIRO, A. E. GHARAVI, B. M. PATEL, P. P. FERJENCIK & G. R. V. HUGHES *Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London*

(Accepted for publication 3 July 1981)

SUMMARY

A microplate radioimmunoassay for IgG, IgA and IgM antiglobulins reactive with rabbit IgG was performed on sera from 69 patients with various connective tissue diseases. IgM antiglobulins were detected in rheumatoid arthritis, systemic sicca syndrome and some patients with systemic vasculitis. IgA antiglobulins were found in seven of 10 patients with systemic sicca syndrome and only five of 59 patients with other connective tissue diseases. There was no correlation between the levels of IgM and IgA antiglobulins in the systemic sicca syndrome or rheumatoid arthritis ($r = -0{\cdot}21$ and 0·2 respectively). IgG antiglobulins were not detected in any serum which lacked IgM or IgA antiglobulins. IgG isolated by DEAE columns showed antiglobulin activity in six of 15 rheumatoid arthritis and two of 10 systemic sicca syndrome sera tested. Antiglobulin analysis of sera fractionated by sucrose density ultracentrifugation at neutral and acid pH enabled the size of each class of antiglobulin to be determined. In certain sera, antiglobulin activity extended into the denser region of the gradient at pH 7·2 suggesting that the antiglobulins were complexed.

INTRODUCTION

IgM rheumatoid factor is almost invariably found in erosive rheumatoid arthritis but is also seen, usually as a transitory phenomenon, in other connective tissue and infectious diseases (reviewed in Hughes, 1979). IgG antiglobulins have been reported to occur in rheumatoid disease (Chodirker & Tomasi, 1963; Hannestad, 1968; Theofilopoulous *et al.*, 1974) and a wide variety of other conditions (Howell *et al.*, 1972; Schur, Bianco & Panush, 1975). Only recently, however, have the technical problems relating to the identification of antiglobulin classes been appreciated (Lea & Ward, 1972; Carson *et al.*, 1977). Using $F(ab')_2$ antibodies to human Fcγ, Pope & McDuffy (1979) demonstrated that IgG rheumatoid factors were rarely found in conditions other than rheumatoid arthritis. With similar techniques, IgA rheumatoid factors were identified in patients with rheumatoid arthritis, Sjögren's syndrome and systemic lupus erythematosus (SLE) (Dunne *et al.*, 1979).

We have recently adapted the antiglobulin solid-phase radioimmunoassay of Hay, Nineham & Roitt (1975) for use in a microplate system taking into account the problems of polyvalent antiglobulins being capable of binding IgG in the patient's serum as well as binding the IgG antibody used to detect antiglobulin class. Since a radioimmunoassay profile of IgG, A and M antiglobulins in the connective tissue diseases has not to our knowledge previously been undertaken, we studied the distribution of antiglobulins in a variety of immunoinflammatory conditions. Our results demonstrate that while none of the antiglobulin classes is specific to any disease, significant differences in the frequency and class distribution of antiglobulins occur.

Correspondence: Dr K. B. Elkon, Department of Medicine, Hammersmith Hospital, London W12 0HS.

0009-9104/81/1200-0547$02.00

MATERIALS AND METHODS

Patients. All patients in the study, except those in the vasculitis groups, had had their disease for at least 2 years. Twenty patients fulfilling the ARA criteria (Ropes *et al.*, 1958) for definite or classical RA were studied. Seven patients had vasculitis associated with rheumatoid disease and the remaining patients were randomly selected from the out-patient clinic. A diagnosis of systemic sicca syndrome was made in 10 patients on the basis of keratoconjuntivitis sicca, xerostomia and systemic complications of which Raynaud's phenomenon, purpura, and arthralgias were the most frequent. None of these patients had erosive arthritis, primary biliary cirrhosis or evidence of another connective tissue disease. A full clinical and immunological description of these patients will be presented (manuscript in preparation). One patient with sicca syndrome secondary to amyloidosis and two patients with idiopathic sicca syndrome but without systemic complications were also studied. Fourteen patients with four or more of the ARA criteria for SLE (Cohen *et al.*, 1971) (eight active and six in remission) and 11 patients with other forms of systemic vasculitis (two polyarteritis nodosa, two Churg–Strauss vasculitis, one Wegener's granulomatosis, three Henoch–Schönlein purpura, one Behçet's syndrome, two essential mixed cryoglobulinaemia) were included. Of the 11 patients with 'seronegative arthritis', two had adult Still's disease, four had arthritis associated with gastrointestinal diseases, one had ankylosing spondylitis and four had a chronic polyarthritis indistinguishable from rheumatoid arthritis except that the latex test for rheumatoid factor had been persistently negative.

The control population consisted of 12 healthy medical personnel (six male, six female) aged between 27 and 56 years (mean 37 years).

Immunoglobulins. Human, rabbit and sheep IgG were purified by 45% saturated ammonium sulphate precipitation and DEAE cellulose (Whatman Ltd, Maidstone, Kent) chromatography in 0·01 M phosphate buffer, pH 8·1. IgM was isolated by thorough washing of a cryoimmunoglobulin M from a patient with Waldenström's macroglobulinaemia. The IgM fraction was obtained from the ascending limb of the exclusion peak on a Sephadex G-200 column (Pharmacia Fine Chemicals, Sweden) at 37°C. Partially purified IgA was prepared from normal human serum by ammonium sulphate precipitation, DEAE cellulose and Sephadex G-200 chromatography. Residual IgG was removed by passage through a Sepharose 4B–protein A column (Pharmacia). Free light chains were prepared by reduction of normal human IgG with 0·02 M dithiothreitol (DTT, Sigma, Missouri, USA) and alkylated with iodoacetamide (Sigma) to a final concentration of 0·12 M. The free light chains were separated by gel filtration on Sephadex G-100 (Pharmacia) in 1·0 M acetic acid.

Antisera. Antisera to human IgG and IgM were raised in New Zealand white rabbits. Sheep antisera to human IgA (α chain, nephelometric grade) were purchased from Seward Immunostics, London, UK. Where appropriate, the antisera were adsorbed with human IgG or light chains covalently coupled to CNBr–Sepharose 4B. All of the antisera were shown to be monospecific by immunoelectrophoresis and double immunodiffusion. In addition, pepsin-digested ^{125}I-labelled IgG fractions (see below) of each antiserum were incubated with *human* IgG adsorbed to the microtitre plate and the percentage binding to IgG calculated.

$F(ab')_2$ fragments were prepared from the IgG fraction of each antiserum by pepsin digestion. In brief, the IgG fraction was dialysed against 0·1 M sodium acetate and the pH adjusted to 3·5. Pepsin (2% w/w) in 0·2 M acetic acid was incubated with the IgG fraction for 2 hr at 37°C. The mixture was neutralized and dialysed against 0·01 M phosphate-buffered 0·15 M saline (PBS) overnight. The $F(ab')_2$ fragment was isolated by gel filtration on Sephadex G-100 in PBS.

The $F(ab')_2$ fragment of IgG was labelled by the chloramine T method (McConahey & Dixon, 1966) to a specific activity of approximately 0·9 μCi/μg.

Microplate antiglobulin radioimmunoassay. The assay was modified from that of Hay *et al.* (1975). Purified whole rabbit IgG at 50 μg/ml was adsorbed to plastic microtitre plates (Flow Laboratories, Scotland) by incubation at 37°C for 1 hr and 4°C overnight. After three washes with PBS/Tween (0·05%), 150 μl of PBS/1% BSA (Sigma) were added to each well and incubated for 2 hr at room temperature. The wells were washed three times with PBS/Tween and duplicate 100-μl aliquots of a 1/10 serum dilution in PBS/BSA added. Incubation was at 37°C for 60 min and 4°C for 30 min. After washing, ^{125}I-labelled $F(ab')_2$ with antibody activity to γ, μ or α chain was incubated

overnight at 4°C. The wells were washed three times with PBS/Tween and counted in a gamma counter (NE 1600, Nuclear Enterprises).

The percentage of ^{125}I-F(ab′)$_2$ binding to each well was compared to the mean percentage binding of the control population. Values greater than 2 standard deviations from the mean were considered abnormal or 'positive'. The results were also expressed as a *binding ratio* which was calculated as follows:

$$\frac{\%\text{ Binding of label to patient serum}}{\text{Mean }\%\text{ binding of label to control sera}}$$

To minimize variation from assay to assay, both the patient sera and labelled antisera were stored in small aliquots at −20°C. Four negative and one positive control sera were included in each run.

To determine the extent to which antiglobulins already bound to rabbit IgG on the microtitre plate were able to bind normal rabbit and sheep IgG or F(ab′)$_2$, the following study was performed. One rheumatoid serum and one systemic sicca serum were applied to microtitre wells in the standard way. In the final step, ^{125}I-labelled normal sheep IgG or F(ab′)$_2$ and normal rabbit IgG or F(ab′)$_2$ were added in concentrations from 5 to 20 μg/ml. After three washes with PBS/Tween, the amount of immunoglobulin bound to the plate was calculated and compared with the amount of F(ab′)$_2$ anti-α and F(ab′)$_2$ anti-μ bound.

IgG antiglobulin assay. For all sera with negative results for IgM and IgA antiglobulins, the assay was performed in the standard way using F(ab′)$_2$ anti-γ. As noted by others (Carson *et al.*, 1977; Pope & McDuffy, 1979), reduction and alkylation of normal sera caused increased IgG binding to the solid phase (see below). We therefore chose to isolate the IgG fraction from sera positive for IgM or A antiglobulins by DEAE minicolumns (Carson *et al.*, 1977) using a 2-ml 50% gel slurry. The IgG fractions were applied to the wells at a concentration of 500 μg/ml PBS/BSA and the antiglobulin assay performed with 1 μg ^{125}I-F(ab′)$_2$ antibody. In six patients and two controls, insufficient IgG was obtained by DEAE chromatography for antiglobulin analysis.

Effect of hyperglobulinaemia, reduction and alkylation and exposure to 0·1 M glycine-HCl, pH 3·0. To assess the effect of a non-specific increase in the serum level of IgA or IgM on the antiglobulin assay, the following patient sera were included in the study (immunoglobulin level in parentheses): IgA myeloma (12·5 mg/ml), SLE (IgA 10 mg/ml), alcoholic cirrhosis (IgA 5·5 mg/ml) and Waldenström's macroglobulinaemia (3·5 and 4·4 mg/ml). To determine whether the enhanced IgG binding in normal sera following reduction and alkylation (using final concentrations of 0·1 M 2-mercaptoethanol and 0·11 M iodoacetamide) or exposure to glycine–HCl (after ultracentrifugation or overnight dialyses in 0·1 M glycine–HCl, pH 3·0, and neutralization with 0·2 M K_3PO_4) was due to binding to rabbit IgG, BSA or the plastic microtitre plate, aliquots of control and treated sera were incubated in wells treated as follows: (a) rabbit IgG and BSA as above, (b) PBS/1% BSA and (c) PBS alone. The percentage of binding of F(ab′)$_2$ anti-IgG to each well was calculated as described above. PBS/BSA to which 2-mercaptoethanol and iodoacetamide were added served as a control for non-specific binding of F(ab′)$_2$ anti-IgG to the microtitre plate.

Ultracentrifugation studies and isolation of an IgA antiglobulin. Ultracentrifugation of rheumatoid arthritis, systemic sicca and normal sera was performed on an MSE 65 prespin ultracentifuge at 150,000 ***g***. Run conditions were 18 hr at 4°C on a linear 10–40% sucrose gradient in PBS, pH 7·2, or 0·1 M glycine–HCl, pH 3·0. Thirty fractions were collected by tube puncture and alternate fractions were further diluted in PBS/1% BSA and tested for antiglobulin activity. Fractions from the acid gradient were neutralized with 0·2 M K_3PO_4 prior to the assay.

To determine the size of the IgA antiglobulins in a patient with systemic sicca syndrome, the patient's serum was incubated with insolubilized normal human IgG (Avrameas & Ternyck, 1969). Antiglobulins were eluted with 0·1 M glycine–HCl pH 2·8, and neutralized with 0·5 M K_3PO_4. IgG and trace amounts of IgM antiglobulin were removed by Sepharose 4B–protein A and Sepharose 4B anti-μ chain respectively. The IgA antiglobulin was labelled with ^{125}I by chloramine T and sucrose density ultracentrifugation performed as above except that a 400-μl cushion of 50% sucrose was included. Prior to ultracentrifugation, one aliquot of the labelled antiglobulin was incubated with heat-aggregated human IgG for 30 min at 37°C. ^{125}I-labelled IgG, C1q (purified by the method of Yonemasu & Stroud, 1971) and IgM were used as 7, 11 and 19S markers respectively.

Statistics. The percentage binding of labelled reagent to normal and patient sera were compared by Student's *t*-test. Correlation coefficients were determined by linear regression.

RESULTS

Quality control experiments

Specificity of antisera. As shown in Fig. 1, only the anti-IgG reagent bound to human IgG. In addition, when sera were fractionated by sucrose density ultracentrifugation at pH 3·0, antiglobulin activity was detected in the 7S (anti-IgG), 11S (anti-IgA) or 19S (anti-IgM) region of the gradient (see Fig. 8).

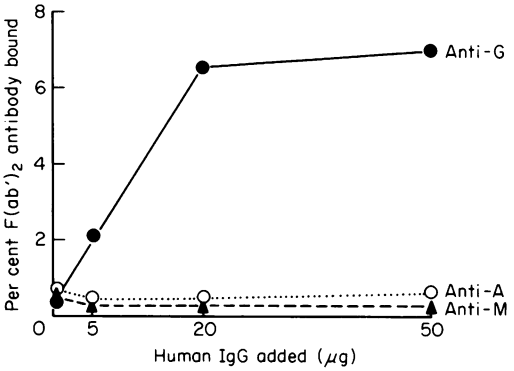

Fig. 1. Percentage binding of ^{125}I-F(ab′)$_2$ anti-IgG (●——●), anti-IgA (o · · · · o) and anti-IgM (▲- - -▲) to human IgG.

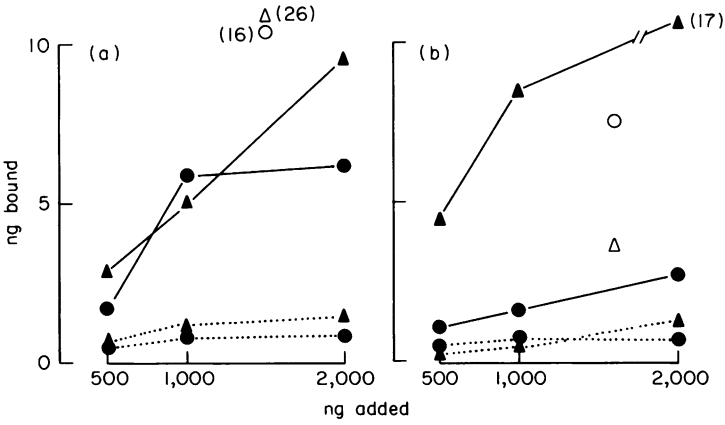

Fig. 2. Binding of normal ^{125}I-IgG (solid lines) and ^{125}I-F(ab′)$_2$ (broken lines) to a rheumatoid (▲) or systemic sicca serum (●) under the conditions of the antiglobulin assay. (a) Rabbit IgG and F(ab′)$_2$ and (b) sheep IgG and F(ab′)$_2$. For comparison, the binding of ^{125}I-F(ab′)$_2$ anti-IgM (a) and ^{125}I-F(ab′)$_2$ anti-IgA (b) to the rheumatoid (Δ) and sicca (O) sera is shown.

Binding of antiglobulins to normal sheep or rabbit IgG. Antiglobulin-containing sera were clearly capable of binding additional normal sheep or rabbit IgG but not the F(ab′)$_2$ fragments (Fig. 2).

Effect of hyperglobulinaemia. As illustrated in Figs 3 & 4, patients with elevated serum IgA or IgM levels had binding ratios either within or just above the normal range. Despite the presence of elevated IgG in many patients in the current study, none were found to have elevated IgG antiglobulins in unfractionated sera (Fig. 5).

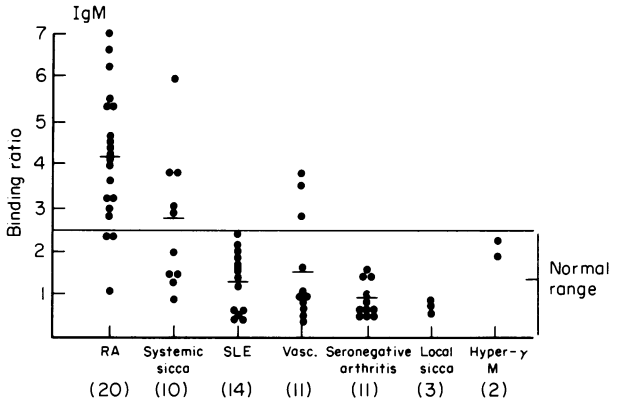

Fig. 3. Binding ratios for IgM antiglobulin in various connective tissue diseases (numbers in each group shown in parentheses). The mean control value is 1·0 and the enclosed area indicates 2 s.d. about this value. The mean binding ratio for each patient group is denoted by a horizontal bar. RA = rheumatoid arthritis, SLE = systemic lupus erythematosus.

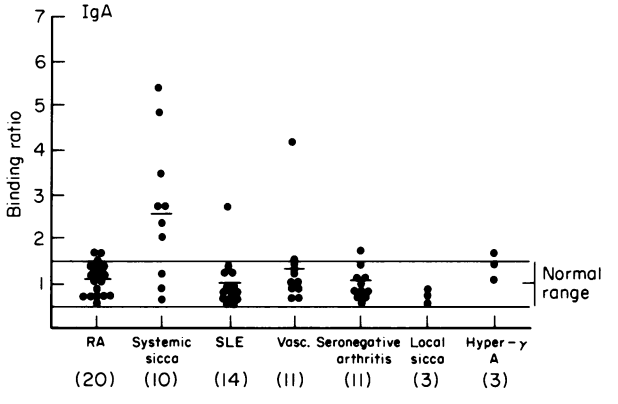

Fig. 4. Binding ratios for IgA antiglobulin. (Key as Fig. 3.)

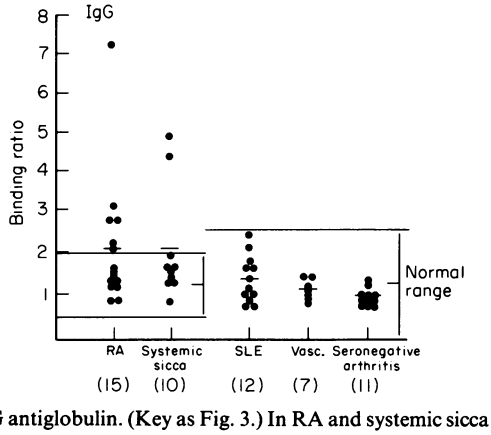

Fig. 5. Binding ratios for IgG antiglobulin. (Key as Fig. 3.) In RA and systemic sicca syndrome, DEAE-purified IgG fractions were tested whereas whole serum was used for other connective tissue diseases.

Exposure of normal serum to reducing agents or low pH. Reduction and alkylation of normal serum caused a significant increase in human IgG binding to the wells regardless of whether the wells were preincubated with rabbit IgG, BSA or PBS alone (Fig. 6). Non-specific binding to the microtitre plate was even higher in serum dialysed in glycine–HCl, pH 3·0 (Fig. 6). Similar results were also seen when serum was reduced with DTT or dialysed against 0·1 M acetate buffer, pH 3·0, and neutralized with 2 M NaOH (results not shown).

Antiglobulin radioimmunoassay

IgM. Significant levels of IgM antiglobulins were demonstrated in patients with rheumatoid arthritis ($P < 0{\cdot}001$) and patients with systemic sicca syndrome ($P < 0{\cdot}05$) (Fig. 3). Patients with RA had significantly higher levels of IgM antiglobulins compared to the group with systemic sicca syndrome ($P < 0{\cdot}02$). Three patients with systemic vasculitis (two essential mixed cryoglobulinaemia and one Wegener's granulomatosis) also had IgM antiglobulins.

IgA. IgA antiglobulin was found in one patient with mixed cryoglobulinaemia, one patient with SLE, two patients with RA and seven of 10 patients with systemic sicca syndrome (Fig. 4). The level of IgA antiglobulin was significantly higher in systemic sicca than in RA ($P < 0{\cdot}01$). There was no correlation between the binding ratios of IgA and IgM antiglobulins in RA ($r = 0{\cdot}2$) or systemic sicca syndrome ($r = -0{\cdot}21$) (Fig. 7).

IgG. IgG antiglobulins were not detected in any of the unfractionated sera of patients with SLE, vasculitis or seronegative arthritis (Fig. 5). Two of 10 patients with systemic sicca syndrome and six of 15 patients with RA (four with vasculitis) had IgG antiglobulins detectable in the IgG fraction isolated by DEAE columns (Fig. 5).

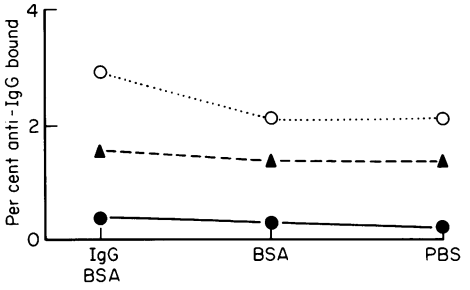

Fig. 6. Percentage binding of F(ab′)$_2$ anti-IgG to normal serum. (●——●) Untreated, (▲— — —▲) reduced and alkylated, (o · · · · o) dialysed against 0·1 M glycine–HCl, pH 3·0. Wells were coated with IgG and BSA, BSA only, or PBS alone. The binding of ^{125}I-F(ab′)$_2$ anti-IgG to wells incubated with 2-mercaptoethanol and iodoacetamide in PBS/BSA was 0·2%.

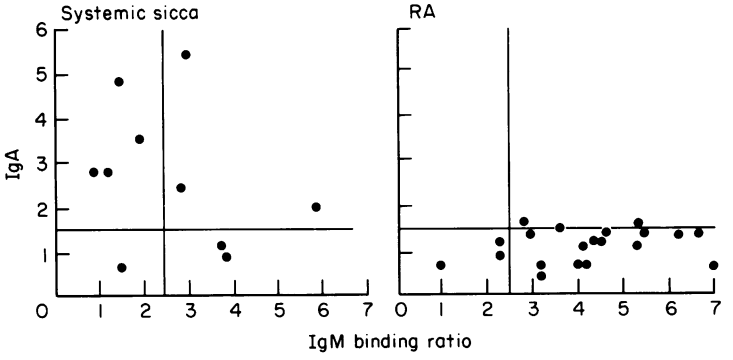

Fig. 7. Binding ratios for IgA antiglobulin (IgA) and IgM antiglobulin (IgM) in patients with systemic sicca syndrome and rheumatoid arthritis. The vertical and horizontal lines represent the normal range.

Ultracentrifugation studies and IgA antiglobulin isolation

Antiglobulin binding of fractionated sera run on a neutral or acid sucrose gradient is shown in Fig. 8. On the acid gradient, elevated IgM antiglobulin activity was demonstrated in the 19S region in the rheumatoid and sicca samples whereas maximal IgA antiglobulin binding was found in the 11S fractions from the patient with systemic sicca syndrome. IgG antiglobulin activity was maximal in the 7S region of the gradient and appeared to be considerably increased compared to serum run at neutral pH in both the patient and normal sera. For IgG, IgA and IgM antiglobulins, activity was also detected in the more dense region of the gradient at pH 7·2 suggesting that the antiglobulins were complexed in the patient sera.

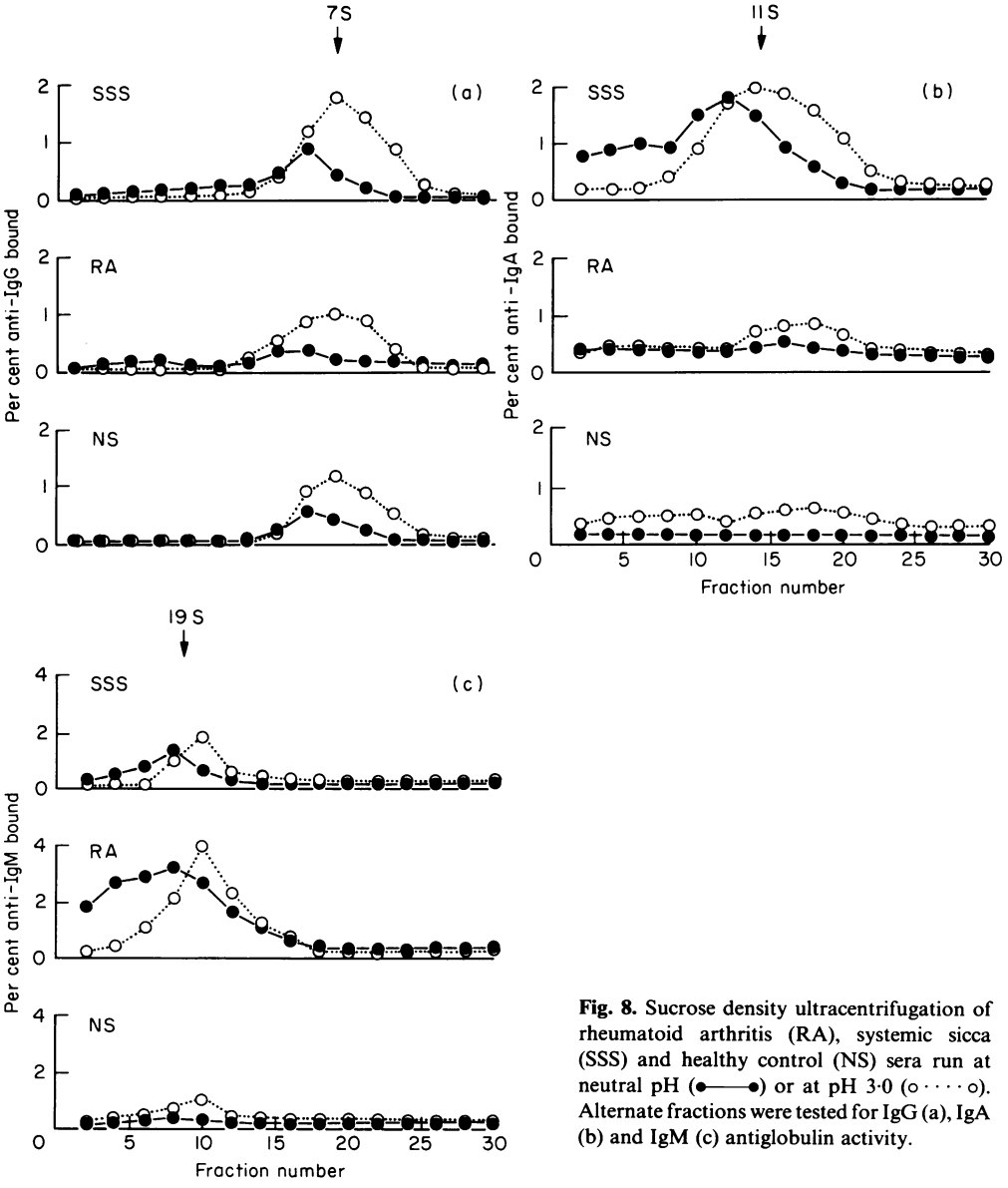

Fig. 8. Sucrose density ultracentrifugation of rheumatoid arthritis (RA), systemic sicca (SSS) and healthy control (NS) sera run at neutral pH (●——●) or at pH 3·0 (o · · · · o). Alternate fractions were tested for IgG (a), IgA (b) and IgM (c) antiglobulin activity.

The IgA antiglobulin isolated from the serum of the patient with systemic sicca syndrome (Fig. 8) reacted with both anti-κ and anti-λ antisera and sedimented in the 11S region of the sucrose gradient (Fig. 9). When incubated with heat-aggregated IgG prior to centrifugation, a significant proportion of the IgA antiglobulin was detected in the sucrose cushion and at the base of the tube (Fig. 9).

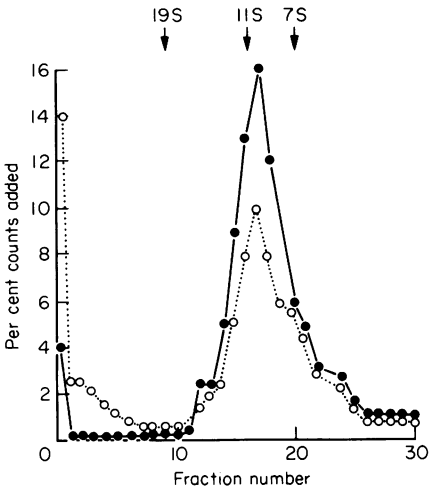

Fig. 9. Isolated and ^{125}I-labelled IgA antiglobulin from the systemic sicca patient shown on Fig. 8. (●——●) Distribution of counts on the sucrose gradient of the antiglobulin, (o · · · · o) effect of prior incubation of the antiglobulin with heat-aggregated IgG. B = base of the centrifuge tube.

DISCUSSION

Although rheumatoid factors were described more than 30 years ago, their role in disease remains controversial. Both detrimental and protective roles have been suggested for IgM (reviewed in Hughes, 1979) whereas IgG rheumatoid factor appears to be more closely related to synovitis (Hannestad, 1968; Munthe & Natvig, 1972) and possibly vasculitis (Theofilopoulous *et al.*, 1974) in rheumatoid disease. The significance of IgA rheumatoid factor (Heimer & Levin, 1966; Abraham, Clarke & Vaughan, 1972; Dunne *et al.*, 1979) is not known. An understanding of the role of antiglobulins in various diseases is dependent upon their accurate detection by sensitive and specific assays. Such assays have only recently been described (Carson *et al.*, 1977; Pope & McDuffy, 1979) and we have modified the original radioimmunoassay of Hay *et al.* (1975) according to these principles. We have confirmed that pepsin digestion of antisera is mandatory for identification of antiglobulin class since antiglobulins bound to solid-phase IgG efficiently bind normal rabbit and sheep IgG but not their respective F(ab′)$_2$ fragments (Fig. 2). The microtitre plate assay described here has advantages in the use of small amounts of reagents as well as low non-specific adsorption of immunoglobulins (Figs 3–5). However, when normal serum was reduced and alkylated or exposed to low pH buffers, a five- to eight-fold increase in non-specific IgG binding to the plastic surface of the microtitre well was observed (Fig. 6). The reagents for the assay (rabbit IgG and F(ab′)$_2$ antisera) are also much simpler to prepare than those described by Carson *et al.* (1977) where human Fc is used as the antigen bound to the solid phase and F(ab′)$_2$ anti-human Fab is used to detect bound rheumatoid factors.

In the present study we have found elevated levels of IgA antiglobulins in seven of 10 patients with systemic sicca syndrome and five of 59 patients with other connective tissue diseases (Fig. 4). Low levels of IgA antiglobulins were detected in only two of 20 patients with erosive rheumatoid arthritis. Recently, IgA rheumatoid factors were detected by radioimmunoassay in patients with rheumatoid arthritis, Sjögren's syndrome and SLE (Dunne *et al.*, 1979). Although the discrepancies

between the two studies may be related to technical differences, the results may also be explained by patient classification. In the current report, patients with local sicca, erosive arthritis or other overlap syndromes were excluded from the systemic sicca group. In addition, all patients (except those with vasculitis) had had their disease for longer than 2 years so that the possibility of evolution into another connective disease was small. In one patient with systemic sicca, the antiglobulin was isolated and shown to be polyclonal dimeric IgA (Figs 8 & 9). Further studies on the nature, origin and significance of IgA antiglobulins are in progress

The findings of significantly elevated IgM antiglobulin in rheumatoid arthritis, systemic sicca syndrome and some patients with other connective tissue diseases (Fig. 3) broadly corresponds with the results obtained by latex fixation or sheep cell agglutination (Hughes, 1979). In addition, the wider range of values observed in the control population aged 27–56 years is consistent with the view that IgM (agglutinating) antiglobulins may occasionally be found in normal subjects (Waller, Toone & Vaughan, 1964).

IgG antiglobulins were not detected in the unfractionated sera of any patient negative for IgM or IgA antiglobulin. In serum fractionated by DEAE columns, IgG antiglobulins were present in six of 15 patients with rheumatoid arthritis and two of 10 patients with systemic sicca syndrome (Fig. 5). Although four of the six patients with IgG antiglobulin had vasculitis, no further attempt was made to determine correlations with disease activity or therapy. Isolation of IgG by ion-exchange DEAE columns was successful for the majority of sera although in six patients and two controls, insufficient IgG was obtained by this method. Whether the IgG isolated is representative of the IgG antiglobulins in the whole serum is unknown since both charge and size may influence elution from the column (Fahey & Terry, 1978). In addition, this method may not be ideal for isolating IgG antiglobulins from cryoglobulin-containing sera unless performed at 37°C.

Antiglobulin analysis of sera fractionated by preparative ultracentrifugation under neutral and dissociating conditions allowed the simultaneous determination of antiglobulin class, size and immune complex formation. Thus antiglobulin activity in the intermediate region of the gradient may be due to polymeric IgA (Figs 8 & 9) rather than, or in addition to, IgG–IgG complexes. These techniques may also be of value in assessing antiglobulin activity of low-molecular-weight (7–8S) IgM (Theofilopoulous *et al.*, 1974; Harisdangkul *et al.*, 1975). In two patients with rheumatoid arthritis and systemic sicca syndrome, antiglobulin activity was found to extend from the 7, 11 or 19S peak into the denser region of the gradient at pH 7·2 (Fig. 8), suggesting that the antiglobulins were, in part, complexed. This pattern was not observed in normal serum nor in the patient serum fractionated under dissociating conditions (Fig. 8). The increase in the peak of antiglobulin activity of the neutralized acid fractions largely reflects an increase in non-specific binding to the microtitre plate (see below).

Although the number of individuals included in each patient group was relatively small, our results suggest that there may be differences in the distribution of antiglobulins in the connective tissue diseases. Whether these differences reflect distinctive alterations in immunoregulation and whether they are related to the clinical manifestations of disease is at present uncertain.

We thank Dr M. B. Pepys for advice and for reviewing the manuscript and Drs J. Goldman and D. Catovsky for supplying serum from patients with lymphoproliferative disorders. We are grateful to the Arthritis and Rheumatism Council for support. Dr P. P. Ferjencik is in receipt of a grant from the Deutsche Forschungsgemeinschaft (No. Fe 180/1).

REFERENCES

ABRAHAM, G.N., CLARK, R.A. & VAUGHAN, J.H. (1972) Characterization of an IgA rheumatoid factor: binding properties and reactivity with the subclasses of human γ G globulin. *Immunochemistry*, **9**, 301.

AVRAMEAS, S. & TERNYCK, T. (1969) The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry*, **6**, 53.

CARSON, D.A., LAWRENCE, S., CATALANO, M.A., VAUGHAN, J.H. & ABRAHAM, G. (1977) Radioimmunoassay of IgG and IgM rheumatoid factors reacting with human IgG. *J. Immunol.* **119**, 295.

CHODIRKER, W.B. & TOMASI, T.B., JR (1963) Low molecular weight rheumatoid factor. *J. clin. Invest.* **42**, 876.

COHEN, A.S., REYNOLDS, W.E., FRANKLIN, E.C., KULKA, J.P., ROPES, M.W., SHULMAN, M.E. &

WALLACE, S.L. (1971) Preliminary criteria for the classification of systemic lupus erythematosus. *Bull. Rheum. Dis.* **21,** 643.

DUNNE, J.V., CARSON, D.A., SPIEGELBERG, H.L., ALSPAUGH, M.A. & VAUGHAN, J.H. (1979) IgA rheumatoid factor in the sera and saliva of patients with rheumatoid arthritis and Sjögren's syndrome. *Ann. Rheum. Dis.* **38,** 161.

FAHEY, J.L. & TERRY, E.W. (1978) Ion exchange chromatography and gel filtration. In *Handbook of Experimental Immunology* 3rd edn (ed. by D. M. Weir). Blackwell, Oxford.

HANNESTAD, K. (1968) Rheumatoid factor reacting with autologous native γ G-globulin and joint fluid γ G aggregates. *Clin. exp. Immunol.* **3,** 671.

HARISDANGKUL, V., MCDOUGAL, J.S., KNAPP, M. & CHRISTIAN, C.L. (1975) Naturally occurring low molecular weight IgM in patients with rheumatoid arthritis, systemic lupus erythematosus and macroglobulinemia. I. Purification and immunologic studies. *J. Immunol.* **115,** 216.

HAY, F.C., NINEHAM, L.J. & ROITT, I.M. (1975) Routine assay for detection of IgG and IgM antiglobulins in seronegative and seropositive rheumatoid arthritis. *Br. Med. J.* **iii,** 203.

HEIMER, R. & LEVIN, F.W. (1966) On the distribution of rheumatoid factors among the immunoglobulins. *Immunochemistry*, **3,** 1.

HOWELL, F.A., CHAMBERLIN, M.A., GERRY, R.A., TORRIGIANI, G. & ROITT, I.M. (1972) IgG antiglobulin levels in patients with psoriatic arthropathy, ankylosing spondylitis and gout. *Ann. Rheum. Dis.* **31,** 129.

HUGHES, G.R.V. (1979) In *Connective Tissue Diseases* 2nd edn. Blackwell, Oxford.

LEA, D.J. & WARD, D.J. (1972) The quantitation of rheumatoid factors. *Arthritis Rheum.* **15,** 646.

MCCONAHEY, P.J. & DIXON, F.J. (1966) A method of trace iodination of proteins for immunologic studies. *Int. Arch. Allergy appl. Immunol.* **29,** 185.

MUNTHE, E. & NATVIG, J.B. (1972) Immunoglobulin classes, subclasses and complexes of IgG rheumatoid factor in rheumatoid plasma cells. *Clin. exp. Immunol.* **12,** 55.

POPE, R.M. & MCDUFFY, S. (1979) IgG rheumatoid factor. Relationship to seropositive rheumatoid arthritis and absence in seronegative disorders. *Arthritis Rheum.* **22,** 988.

ROPES, M.W., BENNETT, G.A., COBB, S., JACOX, R.F. & JESSAR, R.A. (1958) Revision of diagnostic criteria for rheumatoid arthritis. *Bull. Rheum. Dis.* **9,** 175.

SCHUR, P.H., BIANCO, N.E. & PANUSH, R.S. (1975) Antigammaglobulins in normal individuals and in patients with adult and juvenile rheumatoid arthritis and osteoarthritis. *Rheumatology*, **6,** 156.

THEOFILOPOULOUS, A.N., BURTONBOY, G., LOSPALLUTO, J.J. & ZIFF, M. (1974) IgM rheumatoid factor and low molecular weight IgM. An association with vasculitis. *Arthritis Rheum.* **17,** 272.

WALLER, M., TOONE, E.C. & VAUGHAN, E. (1964) Study of rheumatoid factor in a normal population. *Arthritis Rheum.* **7,** 513.

YONEMASU, K. & STROUD, R.M. (1971) C1q: rapid purification method for preparation of monospecific antisera and for biochemical studies. *J. Immunol.* **106,** 304.