

Demonstration of two disease specific antigens in circulating immune complexes

COLETTE DAMBUYANT, JILL BURTON-KEE & J. F. MOWBRAY *Department of Experimental Pathology, St. Mary's Hospital Medical School, London and Clinique Dermatologique, Service du Professeur J. Thivolet, FRA No. 11 'Recherche Dermatologique et Immunologique', Hôpital Edouard Herriot, Place d'Arsonval, 69 374 Lyon, France*

(Accepted for publication 2 March 1979)

SUMMARY

A method is described to assess antigenic cross-reactivity between soluble immune complexes precipitated from sera with polyethylene glycol. The precipitated complex from one serum was dissociated in acid and used to coat a plastic cup. Radioiodinated complexes from another serum were dissociated in the cup, neutralized and allowed to reassociate overnight. The binding of the labelled complex was used to measure the cross-reactivity between the complexes.

Using this technique, complexes from a group of patients with haematuria and hypertension have been found to share an antigen, and a different antigen was found in patients with bullous pemphigoid. The participation of rheumatoid factors in the cross-reactions is unlikely, and no cross-reactivity of either group was found with sera from patients with rheumatoid arthritis.

INTRODUCTION

The detection of circulating complexes in the plasma or serum of patients with immune complex disease is useful in establishing the causal relationship between the complexes and the disease processes, but is only helpful clinically to a limited extent (Zubler & Lambert, 1977). In some diseases the recognition of the antigen causing the immune complex disease has led to the elimination of the antigen, with resolution of the disease (Michael *et al.*, 1966; Koffler, Shur & Kunkel, 1967; Almeida & Waterson, 1969; Treser *et al.*, 1969; Dixon, 1972; Oldstone, 1975; Wands *et al.*, 1975; Carella *et al.*, 1977). Identification of the antigen and antibody specificities in other immune complex diseases is likely to be an essential step towards the elimination of the causal antigen. The work described here is a study of sera for cross-reactions between the antigenic determinants of the circulating immune complexes. Although the nature of the antigens involved is not known, subsets of patients showing the same or a very similar antigen can be found.

MATERIALS AND METHODS

Patients. Twenty-eight adult patients with hypertension attending an out-patient clinic were studied. Haematuria, poor renal function and elevated serum IgA levels were present in some of them. All were receiving hypotensive therapy.

Eighteen patients with bullous pemphigoid (the diagnosis of bullous pemphigoid was based on a positive skin biopsy with linear deposits of IgG and C3 along the basement membrane zone of skin by immunofluorescence (IF) examination) were also included. Indirect IF showed circulating IgG anti-basement membrane zone antibodies in one patient (1:12,500). The ages ranged from 48 to 92 years. Some patients were on corticosteroid therapy.

Controls. As controls thirty skin patients with circulating immune complexes were chosen who did not have bullous pemphigoid. A pool of normal human sera from ante-natal clinics, negative for complexes by our method, was used as the control in the detection of circulating immune complexes using polyethylene glycol (PEG), and individual healthy young

Correspondence: Professor J.F. Mowbray, Department of Experimental Pathology, St. Mary's Hospital Medical School, London W2 1PG.

adult sera were also included. Eight patients with rheumatoid arthritis and circulating immune complexes were also studied.

Detection, preparation and labelling process of the human circulating soluble complexes. Soluble complexes were precipitated with a 2% solution of PEG (polyethylene glycol mol. wt. 6000, Hopkin & Williams Ltd). 0.5 ml volumes were mixed with 0.1 ml of a 12% solution of PEG containing 60 mM EDTA pH 7.6 and were left for 18 hr at 4°C. The precipitate was washed with a 2% solution of PEG containing 10 mM EDTA and was dissolved in 0.5 ml of barbitone-buffered saline pH 7.4. The fraction of the total C1q and IgG which had been precipitated was measured by single radial immunodiffusion (Mowbray, 1976).

The solution of soluble complexes was labelled with ^{125}I (Na^{125}I) (Radiochemical Centre, Amersham), by a modification of the method of Hunter (1973). 100 μl of this solution was mixed with 10 μl of Na^{125}I (1.8×10^7 cpm); chloramine T (BDH Chemicals Ltd.), (50 μg) in 0.5 M phosphate buffer pH 7.5, was added. After 45 sec the reaction was stopped by adding sodium metabisulphite (100 μg) in 0.5 M phosphate buffer pH 7.5. The labelled proteins were separated from iodine excess by dialysing against saline overnight, then an aliquot was precipitated with trichloroacetic acid (TCA) at a final concentration of 5%. The radioactive solution was adjusted at 20–30,000 cpm/10 μl in phosphate-buffered saline (PBS).

Preparation of labelled bovine serum albumin (BSA). 100 μg of radioactive BSA (Sigma Chemical Co.) was prepared by iodination of BSA by using chloramine T with Na^{125}I (Hunter, 1973). The specific activity of the final concentration was approximately 1 mCi/mg.

Preparation of rabbit anti BSA F(ab')₂. Anti-bovine serum albumin serum (anti-BSA-serum) was raised in rabbits, the amount of antibody at the equivalence point being determined by a quantitative precipitation assay. Antibody binding capacity was 0.2 mg BSA/ml of antiserum. BSA antibody complexes were prepared at the equivalence point. The precipitation was allowed to occur for 24 hr at 4°C. The precipitate was centrifuged in the cold at 2000 g for 20 min and washed twice in cold saline. It was then digested with pepsin (pepsin ex hog stomach mucosa, Koch Light Laboratories Ltd.) by Lachmann's method (1971). F(ab')₂ was used without further purification after precipitation with 20% Na_2SO_4 and dialysis against saline. The final F(ab')₂ solution was concentrated ten times.

Preparation of (BSA|rabbit anti-BSA) complexes and labelling. After incubation of a constant amount of anti-BSA serum (0.5 ml) with amounts of BSA ranging from 5 μg (twenty times antibody excess) to 2 mg (twenty times antigen excess) for 24 hr at 4°C, the insoluble complexes were eliminated by centrifugation at 2000 g for 20 min. The supernatant was assumed to contain soluble complexes which were precipitated with a 2% solution of PEG (Mowbray, 1976). Soluble complexes were dissolved in 50 μl of PBS and 10 μl of the solution was labelled with ^{125}I (Na^{125}I) by a modification of the method of Hunter (1973). These complexes were used as either unlabelled or labelled model complexes.

Solid phase cross-reaction technique. This assay involved the adsorption of protein either BSA, F(ab')₂ or complexes after acid dissociation to plastic auto-analyser cups (conical cup LS 127 LK Laboratory Supplies). Buffers used depended on the protein which was being adsorbed to the tubes. For BSA, barbitone-buffered saline pH 7.4 was used; for F(ab')₂ or complexes, 0.1 M Gly-HCl pH 3.1. One ml of these preparations were incubated in plastic cups for 2 hr at room temperature. After three washes with saline, the radioactive material was added in 1 ml of 1% normal horse serum (NHS) (Wellcome Reagents Ltd) or normal rabbit serum (NRS) and left overnight for incubation. If necessary, labelled complexes were dissociated in 0.1 M Gly-HCl pH 3.1 for 20 min, and the solution was then neutralized with $\text{NaOH}/\text{Na}_2\text{HPO}_4$ buffer to pH 7.5 before overnight incubation. Unbound materials were removed by washing three times with saline. The radioactivity in the cups was then counted in a gamma spectrometer (Nuclear Enterprises No. 8311).

RESULTS

Binding of ^{125}I (BSA|anti-BSA complexes) to cups coated with either anti-BSA F(ab')₂ or BSA

10,000–200,000 cpm of labelled soluble or insoluble complexes at different ratios of antigen–antibody (Ag–Ab) were added to cups coated with either the anti-BSA F(ab')₂ solution (1 $\mu\text{l}/\text{ml}$) in 0.1 M Gly-HCl pH 3.1 (Fig. 1) or BSA (2 mg/ml) in barbitone-buffered saline (Fig. 2).

In both experiments, a higher binding of radioactive material was observed with soluble complexes obtained in antigen excess. The low reactivity of soluble complexes in antibody excess suggested a low concentration of protein. With rabbit antibodies most of the complexes in antibody excess are known to be insoluble and a very poor amount of soluble complexes can be obtained. The acid dissociation of the ^{125}I soluble complexes obtained in moderate antigen excess (five to twenty times) gave a rough estimate of the Ag–Ab ratio in the complexes by their ability to react with cold BSA or cold F(ab')₂ adsorbed to cup. Both ^{125}I BSA and ^{125}I IgG were detected in the labelled soluble complexes.

Solid phase cross-reaction technique

Experiment with the model system. The soluble complexes obtained in antigen and antibody excess were used to check the feasibility of the solid phase cross-reaction technique. Cups were not coated either with anti-BSA F(ab')₂ or BSA but with soluble or insoluble complexes. These complexes were disso-

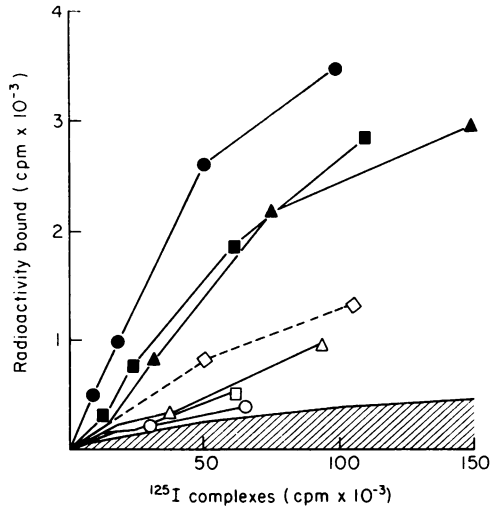


FIG. 1. Binding of ^{125}I BSA/anti-BSA complexes to $\text{F}(\text{ab}')_2$ anti-BSA. Plastic cups were coated with $\text{F}(\text{ab}')_2$ for 2 hr at pH 3.1 and washed. ^{125}I complexes were added in 1% NRS at pH 3.1. After 20 min the pH was raised to 7.5 and the cups left overnight. Antigen excess: 20-fold (\blacktriangle — \blacktriangle); 10-fold (\bullet — \bullet); 5-fold (\blacksquare — \blacksquare); antibody excess: 20-fold (\triangle — \triangle); 10-fold (\circ — \circ); 5-fold (\square — \square); equivalence insoluble complexes (---). The hatched area shows the non-specific background binding.

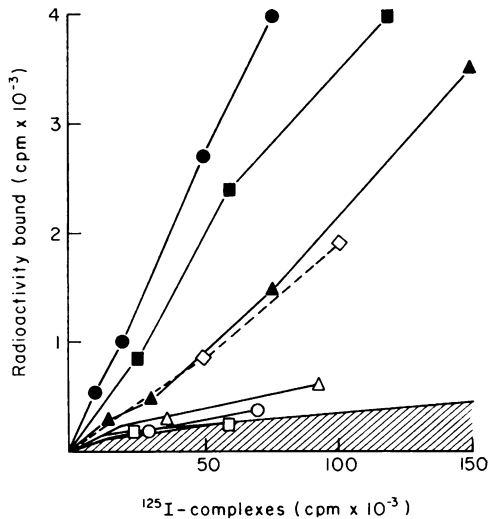


FIG. 2. Binding of ^{125}I BSA/anti-BSA complexes to BSA. Plastic cups were coated with 2 mg/ml BSA for 2 hr and washed. ^{125}I complexes were added in 1% NRS at pH 3.1. After 20 min the pH was raised to 7.5 and the cups left overnight. Antigen excess: 20-fold (\blacktriangle — \blacktriangle); 10-fold (\bullet — \bullet); 5-fold (\blacksquare — \blacksquare); antibody excess: 20-fold (\triangle — \triangle); 10-fold (\circ — \circ); 5-fold (\square — \square); equivalence insoluble complexes (---). The hatched area shows the non-specific background binding.

ciated at acid pH. After 2 hr of coating, the cups were washed and labelled complexes or ^{125}I -BSA were added in 1% NRS at pH 3.1 for 20 min. The solution was then neutralized and left overnight. The results were expressed as a percentage:

$$\frac{\text{radioactivity bound (cpm)} - \text{radioactivity uncoated cup (cpm)}}{\text{radioactivity introduced (cpm)} - \text{radioactivity uncoated cup (cpm)}} \times 100.$$

The results are shown in Table 1.

TABLE 1. Solid-phase cross-reactivity technique applied to the model complexes of BSA/rabbit anti-BSA. Soluble ¹²⁵I complexes at different antigen-antibody ratios were incubated in cups which were coated with the same complexes in acid

¹²⁵ I-labelled complexes	Percentage radioactivity bound to cups pre-coated with:						
	× 20 Ab	× 10 Ab	× 5 Ab	Equivalence	× 5 Ag	× 10 Ag	× 20 Ag
× 20 antibody	0.1	0.2	0.2	0.4	0.6	0.5	0.2
× 10 antibody	0.4	0.5	0.3	0.3	0.6	0.1	0.5
× 5 antibody	0.1	0.2	0.5	0.2	0.5	0.1	0.3
Insoluble complexes at equivalence point	0.2	0.3	0.2	6.4	5.3	3.7	2.2
× 5 antigen	1.1	0.2	0.4	17.8	17.2	14.0	9.8
× 10 antigen	1.9	0.5	0.2	19.7	18.3	15.9	9.1
× 20 antigen	1.4	1.4	0.5	15.1	13.2	9.7	7.5
BSA	3.5	0.6	0.5	67.9	59.6	43.2	25.4



FIG. 3. Solid-phase cross-reactivity method applied to twenty-eight PEG pellets from both immune complex-positive and -negative sera. Positive results are black squares. The cold pellets were first used to adsorb plastic cups (columns), and then ¹²⁵I pellets were added to coated cups (rows).

Soluble complexes in antibody excess were less effective at binding BSA or its antibody than antigen excess complexes (Figs 1 & 2). The high percentage obtained with soluble complexes in antigen excess shows the cross-reaction between the antigenic determinants. In this model, the nature of the antigen was known, and the cross-reaction between the soluble complexes (five-, ten- and twenty-fold excess of antigen) and the bound ¹²⁵I-BSA confirmed the antibody specificity. The higher counts obtained by the addition of ¹²⁵I-BSA could be due either to the efficient adsorption of IgG to the plastic cups or due to a higher specific activity of ¹²⁵I-BSA than ¹²⁵I complexes which contain labelled contaminating proteins.

Experiment with sera of hypertensive patients. Fourteen out of twenty-eight patients had circulating immune complexes, as measured by precipitation by a 2% solution of PEG. The dissolved pellets were used to coat a series of cups (columns in Fig. 3). The same pellets, after iodination, were added to test the cross-reactivity between each (rows in Fig. 3). The percentage binding was measured, a binding over 2% was considered positive. Fig. 3 shows a homogeneous group (Group A) in which the pellets seem to share antigenic determinants or antibody specificity, or a mixture of both. This homogeneous group was selected and tested in the same way with a group of patients with rheumatoid arthritis containing circulating soluble complexes in their sera. No cross-reactivity were found between these sera (Fig. 4).

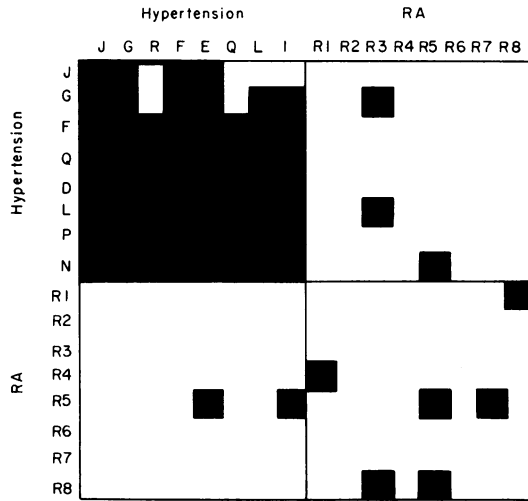


FIG. 4. Solid-phase cross-reactivity method applied to PEG pellets from eight of the hypertensive patients who appeared to share antigenic determinants (cf. Fig. 3) and to PEG pellets from eight immune complex-positive rheumatoid arthritis sera.

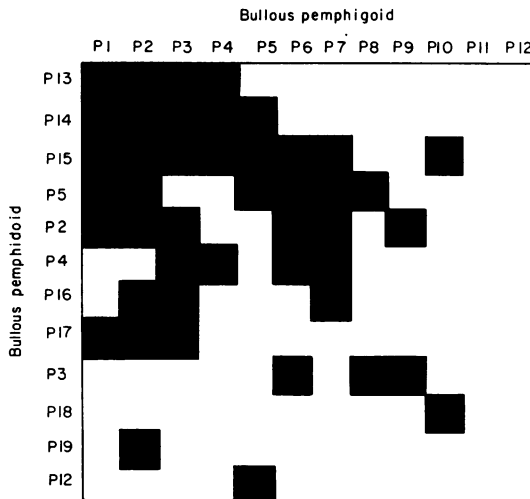


FIG. 5. Solid-phase cross-reactivity method applied to PEG pellets from eighteen bullous pemphigoid patients. Positive results are black squares. The cold pellets were first used to adsorb plastic cups (columns), and the ¹²⁵I pellets were added to coated cups (rows).

Experiment with sera of patients with bullous pemphigoid. The solid phase cross-reaction technique was applied to eighteen sera which came from pemphigoid patients (Fig. 5). The cross-reactivity was not as complete as with the hypertensive group but it was specifically limited to that particular group (Group B).

The serum of one patient with a high titre of circulating IgG anti-basement membrane zone antibodies was used to check the specificity of the complexed antibodies. This serum had a low level of circulating immune complexes as measured by PEG precipitation. Three batches of cups were coated at pH 3.1 and pH 7.4: the first with the whole serum, the second with the dissolved pellet after precipitation by PEG and the third with the supernatant. An iodinated pellet of serum from Group B was added to each of them. A positive result was only found with the whole serum at pH 3 and the pellet at pH 3 and pH 7.4. This experiment suggests that the antibody specificity in the complexes and the anti-basement membrane zone specificity are different.

Other experiments. We found two groups of patients sharing antigenic determinants in their complexes. The antigen(s) is unknown and different as shown in Fig. 6. No cross-reactivity was found between these two groups and various complexes prepared from patients with other skin diseases (disseminated lupus erythematosus, polyarteritis, psoriasis, vasculitis, pyoderma, urticaria, porphyria, etc.).

Attempts were made to test the efficacy of blocking of rheumatoid factor by the use of the horse diluent. Samples from patients with seropositive and seronegative rheumatoid were studied. As Fig. 5 shows there was no consistent cross-reaction observed in this group, either against themselves, or against other members of the group. Similarly, the complexes of the rheumatoid patients did not react with those of the hypertensive patients, although these did cross-react amongst themselves.

DISCUSSION

The technique described here has enabled us to study sera from several diseases in which there are circulating immune complexes, to see whether complexed antigens are shared. A similar study has been published by Delire & Masson (1977), in which the cross-reaction between the antibody in human IgG preparations and that in the circulating immune complexes of patients with idiopathic thrombocytopenic purpura was demonstrated (Lurhuma, Riccomi & Masson, 1977). Indirectly Masson's technique could be used to say that two complexes share the same antigen if they both react with the same IgG preparation. The purpose of their study was different from ours, however, as it was concerned with the effect of transfusion of the patients with the IgG preparations which did cross-react. The method we used is better for studying large numbers of patients in an attempt to assign them to groups sharing a particular antigen. This separation improves the chance of identifying the antigens since if a particular antigen is known for one member of the group it is known for them all. In addition, it provides evidence for or against the common aetiology of a particular disease entity. The two diseases studied here have both shown that the antigen complexed in some people with the disease has not been found in other states studied. This is almost certainly by chance, since it is known that the same antigen may be found complexed in different diseases. A good example of this is the identification of hepatitis B surface antigen in the complexes of some patients with glomerulonephritis, polyarteritis nodosa and mixed cryoglobulin-aemic purpura (Gocke *et al.*, 1970; Trepo & Thivolet, 1970; Combes *et al.*, 1971; Trepo *et al.*, 1974; Levo *et al.*, 1977). Obviously, the demonstration that the same antigen is complexed in patients with the same disease does not mean that the complexes cause the disease, but it may nevertheless help to reveal a common causal infectious agent in some diseases, which may be amenable to treatment.

A method which we have described previously allowed the demonstration of the antigenic specificity of the complex but did not enable us to study cross-reactivity between two sera, unless the specific antigen was available (Dambuyant, Burton-Kee & Mowbray, 1978). In addition, it was not possible to use reference antibodies only in the technique. The current method can be used not only to demonstrate the cross-reaction between two different complexes from different sera, but the cups can be coated with either specific antigen or antibody preparations to identify the specific antigen. Most of the methods used previously for the identification of the antigen in immune complexes in either serum or tissues

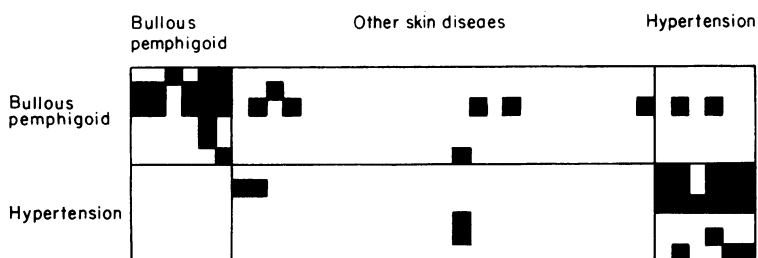


FIG. 6. Solid-phase cross-reactivity method applied to PEG pellets from six patients (Group A, Fig. 3) PEG pellets from six bullous pemphigoid patients (Group B, Fig. 5), and to thirty PEG pellets from circulating immune complex-positive patients with other skin diseases.

have, with the exception of that of Masson, used antibody to detect free antigenic determinants in the complex. Some have eluted specific antibody from complexes in tissues and tested these by reaction against their specific antigens, for example eluted anti-DNA in renal biopsies in DLE (Koffler *et al.*, 1967). The present method is applicable for use with either reference antigen or antibody, and we have shown that it will work when the complex is in either antigen or antibody excess. In antibody excess, the model system did not bind labelled BSA well, but it is known to be difficult to obtain antibody excess BSA complexes in solution without a large excess of antibody. This artefactually diminishes the binding. With antibody excess complexes occurring in serum this effect may be much less. The major disadvantage is that where the antigen or antibody sites are acid labile the technique will fail to demonstrate a cross-reaction. The use of a higher pH for dissociation may overcome this for some antigens, but the efficiency of dissociation is greatly reduced when the pH is raised. We believe that we may have observed an example of this in an unpublished finding that in the complexes of transfused haemophiliac patients, pepsin treatment of the complex liberated a F(ab')₂ with anti-coagulant factor VIII activity, but cross-reaction between the sera with this complex was not observed in the solid phase assay using acid dissociation as described. The antigen of factor VIII is known to show considerable acid lability.

The most common shared antigens in immune complexes which would be expected to show a cross-reaction would be those antigens on immunoglobulins to which anti-antibody and rheumatoid factors are directed. Preliminary studies have shown that these represented a considerable obstacle when attempting to measure a cross-reaction for other antigens in immune complexes. We therefore used horse serum in the assay as a diluent in order to compete with the human immunoglobulin in the complexes for the rheumatoid factors present. The finding that rheumatoid complexes did not show a material cross-reaction with themselves or with other complexes demonstrated that the blockage was effective. It is of interest that some reactivity among certain rheumatoid arthritis complexes remains, possibly of another antigen. Further study is required to eliminate the possibility that residual rheumatoid factor activity, or even an anti-idiotypic antiglobulin is not responsible.

It might be expected that complexes, on reassembly, would react with themselves; Fig. 3, however, shows that this is not always the case. For complexes to be soluble they must be in antigen or antibody excess; complexes which are good at coating cups with predominantly either antigen or antibody may be relatively deficient at binding in sites of the opposite kind. There is some evidence from experiments on artificial complexes that the cross-reaction is dependent on the antigen-antibody ratio, although in the results presented here there is an apparent negative binding of complexes with themselves in the hypertension/haematuria group, this is not so true of the bullous pemphigoid sera. In addition, in other studies (Burton-Kee, Lehner & Mowbray, 1979) we have found good reactions of complexes with themselves in patients with Behçet's disease. Thus, although the presence or absence of self-reactivity of complexes may be dependent on their composition, it is not sufficiently clear as yet to be used to assess any compositional factors in the complex.

The two groups of patients in which we have shown antigenic cross-reaction specific for the disease are not amongst those in which circulating complexes are normally considered to play a role in the expression of the disease. In the hypertensive patients, however, the group with complexes showing a cross-reaction were concentrated in those patients with haematuria, renal failure and elevated IgA levels. This group would be expected to include some of the adult type of IgA/C3 disease described by Berger (Berger, 1969; Berger & Hinglais, 1968). The association with clinical features of this group will be published in greater detail elsewhere. A fraction of such glomerulonephritic patients may present with hypertension, especially in the group described by Berger, in which proteinuria is slight. It is possible that the antigenic cross-reacting group of hypertensives are actually a subgroup with IgA/C3 disease in the adult form, with hypertension, continuous microscopic haematuria and renal failure (Berger, 1969; Berger & Hinglais, 1968; Druet *et al.*, 1970; Droz, 1975).

Cross-reacting complexes circulating in patients with bullous pemphigoid may be explained by the basement membrane zone antigen being complexed with the antibody found in the circulation of many of these patients (Jordon *et al.*, 1967; Chorzelski *et al.*, 1968; Peck *et al.*, 1968). We have been unable to study this possibility with the present technique, but the use of the preparation of a pepsin F(ab')₂ from

the complex failed to produce detectable antibody from the complex. The fact that high titre antibody containing sera will only coat the cup so that complexes will bind when the coating is done at acid pH suggests that the free antibody is of a different specificity from that present in the complex. The nature of the complexed antibody therefore requires further study.

We feel that the preliminary approach described here may enable a more rational approach towards the identification of the antigens of infectious aetiology causing immune complex disease and their elimination by classical therapeutic means or by immunotherapeutic techniques such as that of Masson (Delire & Masson 1977).

The authors would like to thank Professor W.S. Peart, St. Mary's Hospital, and Professor J. Thivolet, Hôpital Edouard Herriot, Lyon, for providing the sera, and Mrs H. Wealleans for valuable secretarial assistance. This study was supported by grants from the Institut National de la Santé et de la Recherche Médicale, the Medical Research Council, The Chest and Heart Association and UER de Biologie Humaine, Université Claude Bernard, Lyon.

REFERENCES

- ALMEIDA, J.D. & WATERSON, A.P. (1969) Immune complexes in hepatitis. *Lancet*, ii, 983.
- BERGER, J. (1969) IgA glomerular deposits in renal diseases. *Transplant. Proc.* 1, 939.
- BERGER, J. & HINGLAIS, N. (1968) Les dépôts intercapillaires d' IgA-IgG. *J. Urol. Nephrol.* 74, 694.
- BURTON-KEE, J., LEHNER, T. & MOWBRAY, J.F. (1979) Circulating immune complexes of Behçet's disease. *Proceedings of a Multidisciplinary Symposium on Behçet's Syndrome* (ed. by T. Lehner and C.G. Barnes). Academic Press (in press).
- CARELLA, G., DIGEON, M., FELDMANN, G., JUNGERS, P., DROUET, J. & BACH, J.F. (1977) Detection of hepatitis B antigen in circulating immune complexes in acute and chronic hepatitis. *Scand. J. Immunol.* 6, 1297.
- CHORZELSKI, T.P., JABLONSKA, S., BLASZCZYK, M. & JARZABEK, M. (1968) Autoantibodies in pemphigoid. *Dermatologica*, 136, 325.
- COMBES, B., STATSNY, P., SHOREY, J., EIGENBRODT, E.H., BARRERA, A., HULL, A.R. & CARTER, N.W. (1971) Glomerulonephritis with deposition of Australia antigen antibody complexes in glomerular basement membrane. *Lancet*, ii, 234.
- DAMBUYANT, C., BURTON-KEE, J. & MOWBRAY, J.F. (1978) The use of the preparation of F(ab')₂ antibody from soluble immune complexes to determine the complexed antigens. *J. Immunol. Methods* 24, 31.
- DELIRE, M. & MASSON, P.L. (1977) The detection of circulating immune complexes in children with recurrent infections and their treatment with human immunoglobulins. *Clin. exp. Immunol.* 29, 385.
- DIXON, F.J. (1972) Pathogenesis of immunologic disease. *J. Immunol.* 109, 187.
- DROZ, D. (1975) Natural history of primary glomerulonephritis with mesangial deposits of IgA. *Clin. Nephrol.* 4, 165.
- DROUET, P., BARIETY, J., BERNARD, D. & LAGRUE, G. (1970) Les glomérulopathies primitives à dépôts mésangiaux d' IgA et d' IgG. Etude clinique et morphologique de 52 Cas. *Presse Méd.* 78, 583.
- GOCKE, D.J., HSU, K., MORGAN, C., BOMBARDIERI, S., LOCKSHIN, M. & CHRISTIAN, C.L. (1970) Association between polyarteritis and australia antigen: a new association. *Lancet*, ii, 1149.
- HUNTER, W.M. (1973) *Handbook of Experimental Immunology* (ed. by D.M. Weir), p. 171. Blackwell, Oxford.
- JORDON, R.E., BEUTNER, E.H., WITEBSKY, E., BLUMENTAL, G., HALE, W.L. & LEVER, W.F. (1967) Basement zone antibodies in bullous pemphigoid. *J.A.M.A.* 200, 751.
- KOFFLER, D., SHUR, P.H. & KUNKEL, H.G. (1967) Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. exp. Med.* 126, 607.
- LACHMANN, P.J. (1971) The purification of specific antibody as F(ab')₂ by the pepsin digestion of antigen-antibody precipitates and its application to immunoglobulin and complement antigens. *Immunochemistry*, 8, 81.
- LEVO, Y., GOREVIC, P.D., KASSAB, H.J., ZUCKER-FRANKLIN, D. & FRANKLIN, E.C. (1977) Association between hepatitis B virus and essential mixed cryoglobulinemia. *N. Engl. J. Med.* 296, 1501.
- LURHUMA, A.Z., RICCOMI, H. & MASSON, P.L. (1977) The occurrence of circulating immune complexes and viral antigens in idiopathic thrombocytopenic purpura. *Clin. exp. Immunol.* 28, 49.
- MICHAEL, A.F., DRUMMOND, K.N., GOOD, R.A. & VERNIER, R.L. (1966) Acute post-streptococcal glomerulonephritis; immune deposit disease. *J. clin. Invest.* 45, 237.
- MOWBRAY, J.F. (1976) *Cours International de Transplantation et d' Immunologie Clinique*, (ed. by J.D. Touraine), p. 35. Simep, Lyon.
- OLDSTONE, M.B.A. (1975) Virus neutralization and virus-induced complex disease: virus antibody union resulting in immunoprotection or immunologic injury—two sides of the same coin. *Progr. Med. Virol.* 19, 84.
- PECK, S.M., OSSERMAN, K.E., WEINER, L.B., LEFKOVITS, A. & OSSERMAN, R.S. (1968) Studies in bullous diseases: Immunofluorescent serologic tests. *New Engl. J. Med.* 279, 951.
- TREPO, C. & THIVOLET, J. (1970) Hepatitis associated antigen and periarteritis nodosa. *Vox Sang.* (Basel), 19, 410.
- TREPO, C., ZUCKERMAN, A.J., BIRD, R.C. & PRINCE, A.M. (1974) The role of circulating hepatitis B antigen-antibody immune complexes in the pathogenesis of vascular and hepatic manifestation in polyarteritis nodosa. *J. clin. Path.* 27, 863.
- TRESER, G., SEMAR, M., MCVICAR, M., FRANKLIN, M., TY, A., SAGEL, I. & LANGE, K. (1969) Antigenic streptococcal components in acute glomerulonephritis. *Science*, 163, 676.
- WANDS, J.R., MANN, E., ALPERT, E. & ISSELBACHER, K.J. (1975) The pathogenesis of arthritis associated with acute hepatitis B surface antigen hepatitis: complement activation and characterization of circulating immune complexes. *J. clin. Invest.* 55, 930.
- ZUBLER, R.H. & LAMBERT, P.H. (1977) Immune complexes in clinical investigation. *Recent Advances in Clinical Immunology* (ed. by R.A. Thompson), p. 125. Churchill Livingstone, Edinburgh.