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Isolation and characterization of circulating immune complexes in cystic fibrosis

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SUMMARY

A methodology for the isolation and immunologic characterization of IgG-containing circulating immune complexes (IgG-CIC) as detected by the ^{125}I-C1q-binding assay (C1qBA) is described. We applied this methodology to sera from patients with cystic fibrosis (CF), both positive and negative for IgG-CIC. We used latex-fixation-positive rheumatoid arthritis sera and normal human sera as positive and negative controls, respectively. All IgG-CIC-positive serum samples from CF patients were found to contain antibodies against *Pseudomonas aeruginosa* in the isolated complexes. Some patients also had antibodies in CIC specific for *Staphylococcus aureus* and *Candida albicans*. CIC specificity corresponded to respiratory tract colonization for each patient.

INTRODUCTION

Circulating immune complexes (CIC) have been detected in many diseases characterized by persistent antigenic stimulation. Sources for these antigenic stimuli include exogenous infective agents, neoantigens associated with malignant transformation, and self-antigens which normally do not provoke an immune response. In many of these diseases, pathophysiological changes have been ascribed to the CIC through their ability to induce inflammatory reactions. The pathogenicity of CIC is a function of a number of factors such as valence, antigen–antibody ratio, affinity, physicochemical properties of the antigen and antibody, function of the reticuloendothelial clearance system, and interactions with humoral and cellular ligands (Theofilopoulos & Dixon, 1979; Zubler & Lambert, 1977; Haakenstad & Mannik, 1977).

In some diseases the identification of antigen–antibody characteristics of CIC is facilitated by precise knowledge of their pathogenesis as, for example, in a chronic infectious disease where a single agent is suspected of providing the antigen(s) found in disease-associated CIC (Bjorvatn *et al.*, 1976; Harkiss, Brown & Evans, 1979; Pernice *et al.*, 1979; Casali & Lambert, 1979; Yoshinoya, Cox & Pope, 1980; Tabbarah *et al.*, 1980; Anh-Tuan & Novak, 1981). In many diseases with CIC, however, the cause is obscure and the detection of CIC by a wide variety of antigen-non-specific techniques (Lambert *et al.*, 1978) does little to give further insight into the underlying pathologic process. The isolation of CIC and identification of antigen and antibody in the CIC represent important advances in relating the CIC to the basic disease.

An example of a disease where CIC are often detected while the underlying pathophysiology remains unknown is cystic fibrosis (CF) (Schiotz *et al.*, 1977; Moss & Lewiston, 1980; Berdischewsky *et al.*, 1980). We have related the presence and levels of CIC to exacerbations of the

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lung disease that is characteristic of CF, a finding that suggests their pulmonary origin and involvement in lung damage (Moss, Hsu & Lewiston, 1981). Pulmonary origin of CIC in CF is further suggested by findings of immune complexes and activated complement components in the pulmonary compartment without systemic complement activation (Moss & Lewiston, 1980; Schiotz *et al.*, 1978; Schiotz, Sørensen & Hoiby, 1979), and by deposition of immunoglobulin and complement in lung tissue of CF necropsy specimens (McFarlane *et al.*, 1975).

We describe in this report a reliable method for isolating CIC from serum and characterizing the antibody specificities. Sera containing CIC were identified by the ^{125}I-C1q-binding assay (Zubler & Lambert, 1976). After 50% ammonium sulfate precipitation, CIC were separated from monomeric IgG by gel filtration on Sephadex G150. The macromolecular peak was applied to protein A-Sepharose and the retained IgG-CIC eluted at acid pH. Isolated IgG-CIC were then dissociated with 9 M urea and fractionated on a second, smaller Sephadex G150 column into peaks positive and negative for IgG on Ouchterlony analysis. The IgG-positive peak was tested for antibody specificity against a battery of possible relevant antigens in a microtitre solid-phase radioimmunoassay (MSPRIA) (Moss & Lewiston, 1980).

METHODS

Patients. Patients with cystic fibrosis diagnosed according to standard criteria (Wood, Boat & Doershuk, 1976) were screened for CIC by the ^{125}I-C1q-binding assay of Zubler and Lambert (Zubler & Lambert, 1976). Routine bacteriology was done on expectorated sputum. Rheumatoid arthritis serum was obtained from a pool of seropositive adults which had > 50% ^{125}I-C1q binding and latex fixation titre > 1:256. Normal human serum was obtained from laboratory personnel with ^{125}I-C1q binding of < 8%. Serum *Pseudomonas*-specific IgG antibody was determined by radioimmunoassay as described previously (Moss *et al.*, 1981).

Ammonium sulfate precipitation. Three millilitres of test serum was incubated 1:1 (v/v) with a saturated solution of $(NH_4)_2SO_4$ for 1 hr at 4°C. The precipitate was dissolved in 2–3 ml of 0·01 M phosphate-buffered saline (PBS), pH 7·2, and dialyzed overnight at 4°C against 1 litre PBS with one change of buffer.

Sephadex gel filtration. The resuspended, dialyzed gamma globulin fraction was applied at room temperature to a Sephadex G150 column (2·4 × 60 cm) and eluted with PBS–0·1%-sodium azide at a downward flow rate of 15 ml hr^{-1}. Fractions of 0·875 ml per tube were collected automatically. Protein content was obtained spectrophotometrically and immunoglobulin content assayed by double immunodiffusion or, where appropriate, by single radial immunodiffusion (Calbiochem-Behring, La Jolla, California). Rheumatoid factors were assayed by latex fixation (Hyland Diagnostics, Deerfield, Illinois).

Affinity chromatography. The macromolecular peak (see Results later) of the Sephadex G150 filtrate was pooled, perevaporated to a final volume of 3 ml, and applied at room temperature to a 1.7 × 3 cm staphylococcal protein A-Sepharose CL-4B column (Sigma Chemical Co., St Louis, Missouri). After saturation of the Sepharose, flow was stopped for 30 min. Elution was then carried out with 0·1 M sodium phosphate, pH 7·0, and 1-ml fractions collected until protein content was down to background levels (< 0·02). The column was then eluted with 1 M acetic acid, pH 2·5, and again fractions collected until protein content of the eluate reached background levels. Neutral and acid eluates were tested for immunoglobulin content and rheumatoid factor activity as described earlier. Immediately after collection the acid eluate was dialyzed overnight against PBS–0·1%-sodium azide at 4°C with two buffer changes, followed by perevaporation to a final volume of 1 ml.

Dissociation of isolated CIC. The perevaporated acid eluate was incubated with 9 M urea, pH 7·4, at room temperature for 4 hr and then immediately applied to a second, smaller (1·4 × 85 cm) Sephadex G150 column, and eluted with PBS–azide at a flow rate of 15 ml hr^{-1} in 0·875 ml-fractions (3·5 min per tube). The protein peaks obtained were each pooled for immunoglobulin and rheumatoid factor testing as described.

Immunologic characterization of isolated, dissociated CIC. The IgG-positive peak from the second G150 column was diluted in 10% normal goat serum (Gibco Laboratories, Santa Clara,

California) at serial ten-fold dilution of 10^{-1} to 10^{-3}. Aliquots were then assayed against a nine-antigen battery in a MSPRIA as described previously (Moss & Lewiston, 1980). The antigens employed in the MSPRIA were: 1. *Pseudomonas aeruginosa* heptavalent lipopolysaccharide antigen (Hanessian *et al.*, 1971) (PA LPS) courtesy of Dr M. S. Fisher (Parke-Davis, Detroit, Michigan, lot X-41757). 2. *Pseudomonas aeruginosa* ultrasonicate (PA SON) prepared in our laboratory from five CF mucoid isolates as described previously (Moss *et al.*, 1981). 3. *Staphylococcus aureus* ultrasonicate (SA) similarly prepared from a culture of a protein-A-deficient strain (Wood 46) (American Type Culture Collection, Rockville, Maryland, lot 10832). 4. *Candida albicans* allergenic extract (CA) (Hollister-Stier Laboratories, Spokane, Washington, lot JO3311M). 5. *Aspergillus fumigatus* hypersensitivity pneumonitis antigen (AF) (Hollister-Stier Laboratories, Spokane, Washington, lot L10911504). Binding of this antigen to the solid phase was enhanced by prior conjugation to poly-L-lysine with cyanuric chloride (Gray, 1979). Both fungal extracts were extensively dialyzed against PBS before use. 6. Bovine trypsin type III (Sigma Chemical Co., St Louis, Missouri, lot 80F-8065). 7. Human colostral secretory IgA (sIgA), courtesy of Dr J. O'Leary, Santa Clara Valley Medical Center, San Jose, California. Ouchterlony and immunoelectrophoretic analysis of the preparation showed purity except for trace contamination with lactoferrin. 8. CF bronchopulmonary lavage fluid; obtained at therapeutic bronchoscopy. Saline lavage material was centrifuged and the cell-free supernatant used after sterilization with gentamicin and sodium azide. 9. Human purified IgG (Miles Laboratories, Elkhart, Indiana).

Antigens were used at a final protein concentration of 20 μg ml^{-1} (Lowry *et al.*, 1951). Solid-phase binding of antigens was established in preliminary experiments by trace radiolabelling using the chloramine T method (Greenwood & Hunter, 1963). For the MSPRIA, 50 μl per well of antigen was added to 96-well polyvinylchloride microtitre plates (Cooke Laboratories Products, Alexandria, Virginia). The MSPRIA was performed as described previously (Moss *et al.*, 1981) except that the isolated CIC were diluted in 10% normal goat serum (Gibco, Santa Clara, California) and incubated overnight. A positive response was defined as a titration curve showing >1500 counts min^{-1} binding at 10^{-1} decreasing $>95\%$ at 10^{-3}.

Table 1. Respiratory colonization and immune responses

Subject	Diagnosis	Colonization	C1qBA (%)	IgG (mg dl^{-1})	IgA	IgM	IgM-RF (titer)	PA IgG (net counts min^{-1})
1	CF	PA	47·0	2,500	230	280	—	19,948
2	CF	PA, CA, *Proteus mirabilis*	32·3	1,550	550	81	—	19,087
3	CF	PA, CA	28·0	2,200	490	127	+ 1:8	17,183
4	CF	CDC Group VE-1*	57·6	2,000	550	287	—	9,307
5	CF	PA	7·2	1,525	205	87	—	12,791
6	N1	N1 Flora	7·0	1,125	280	73	—	1.184
7	RA	ND	52·0	1,275	375	350	+ >1:256	1,605

* Uncertain taxonomy; Center for Disease Control classification; a Gram-negative rod resembling *Herrelia* spp.

C1qBA = ^{125}I-C1q-binding assay; IgM-RF = latex-fixation-positive rheumatoid factor; PA IgG = *Pseudomonas aeruginosa*—specific serum IgG; net counts min^{-1} = counts min^{-1} antigen wells − counts min^{-1} filler wells (mean of duplicates) in solid-phase radioimmunoassay).

CF = cystic fibrosis; N1 = normal subject; RA = adult seropositive rheumatoid arthritis serum pool; CA = *Candida albicans*. ND = not done.

RESULTS

Patients

Sera from four CF patients with high ^{125}I-C1q binding (CIC⊕) were studied and compared to normal serum, pooled sera from adult seropositive rheumatoid arthritis patients, and a CF patient with normal ^{125}I-C1q binding (CIC⊖) who was colonized with PA and had comparable levels of PA IgG to the CIC-⊕ patients. Colonization and immune response information on the patients is listed in Table 1.

Sephadex G150 gel filtration

The elution profiles of the chromatographed ammonium sulfate precipitates for normal serum, RA serum and CF CIC⊕ and CIC⊖ sera are shown in Fig. 1. As previously reported (Chenais *et al.*, 1977), CIC⊖ sera (here, both in normal and CF subjects) exhibited distinct protein peaks which discriminated monomeric IgG from IgM under our filtration conditions. In contrast, CIC⊕ sera (here, both RA and CF) showed extension of IgG on Ouchterlony analysis into the macromolecular tubes and only one protein peak.

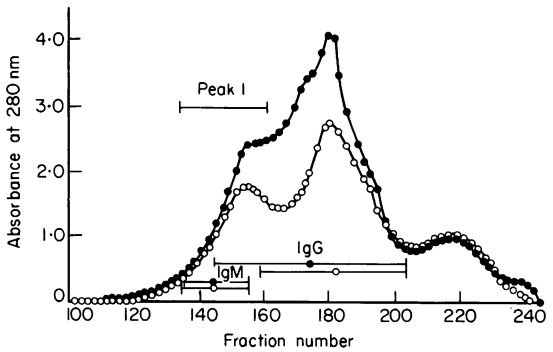

Fig. 1. Elution profiles for gel filtration on Sephadex G150 of $(NH_4)_2SO_4$-precipitated protein in CF CIC⊕ patient no. 1 (●) and CF CIC⊖ patient no. 5 (○). Fractions containing IgG and IgM are also shown for both sera. Other CF CIC⊕ and the RA sera profiles were similar to that shown by no. 1, while normal serum, no. 6, was similar to that shown by CF, no. 5.

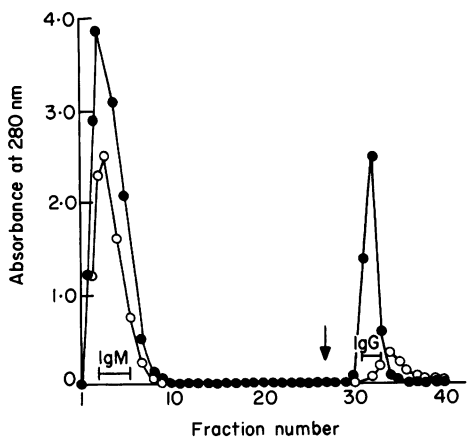

Fig. 2. Affinity chromatography on protein A-Sepharose of peak 1 from Sephadex G150 filtration of CF CIC⊕ patient no. 3 (●) and CF CIC⊖ patient no. 5 (○). The arrow indicates beginning elution with 1 M acetic acid, pH 2·5. IgG was found in acid eluates of CIC⊕ sera. IgM and small amounts of IgG were found in neutral eluates of all sera. Other CF CIC⊕ sera and the RA sera were similar to no. 3; normal subject no. 6 was similar to no. 5. In the RA pool latex fixation was found mainly in the neutral eluate, but also weakly in the acid eluate.

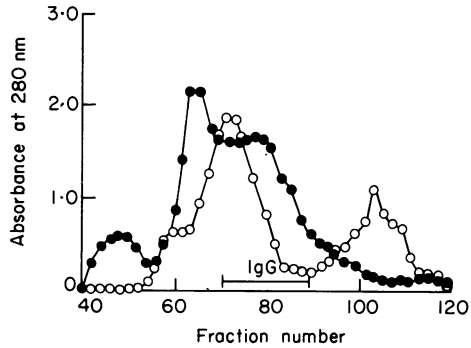

Fig. 3. Elution profiles on Sephadex G150 of protein A-Sepharose acid eluates from RA no. 7 (•) and CF CIC⊕ no. 2 (o). IgG was found in all CIC⊕ samples in fractions 70–90, which were then used for detection of antibody specificity in the MSPRIA. In the RA pool latex fixation was detected in fractions 60–70, and a precipitin line formed between perevaporated concentrates of fractions 60–70 and 70–90 on Ouchterlony analysis.

Table 2. Titration curve data for antibody specificities in isolated CIC or equivalent fractions

		Net counts min^{-1} for subject number						
Antigen*	CIC dilution	1	2	3	4	5	6	7
PA SON	10^{-1}	10,562	11,956	7,585	1,877	73	125	288
	10^{-2}	7,126	5,888	1,381	290	9	26	187
	10^{-3}	3,112	665	278	−380	34	77	166
PA LPS	10^{-1}	4,762	9,956	12,330	38	153	79	143
	10^{-2}	1,278	5,284	4,668	5	−197	99	77
	10^{-3}	413	585	1,111	162	183	65	26
SA	10^{-1}	7,593	1,125	775	237	−209	19	170
	10^{-2}	9,030	360	144	100	−71	27	72
	10^{-3}	388	112	28	59	−6	102	44
CA	10^{-1}	271	5,170	2,499	105	−21	68	495
	10^{-2}	85	1,057	452	34	87	56	195
	10^{-3}	409	343	145	112	50	190	160
AF	10^{-1}	308	481	699	256	93	151	78
	10^{-2}	5	−9	123	138	629	40	−28
	10^{-3}	163	−94	550	163	321	41	215
Trypsin	10^{-1}	137	1,143	600	571	40	−14	27
	10^{-2}	3	1,127	427	455	157	37	5
	10^{-3}	32	1,266	461	−189	74	56	20
sIgA	10^{-1}	2,409	1,456	369	265	163	−62	487
	10^{-2}	2,950	1,287	272	657	320	134	355
	10^{-3}	2,700	1,193	27	324	247	−9	189
CF lavage	10^{-1}	3,575	7,550	2,126	927	308	248	297
fluid	10^{-2}	2,658	4,794	2,379	1,536	345	305	338
	10^{-3}	2,737	2,980	1,899	1,553	314	311	240
IgG	10^{-1}	16,165	20,468	16,552	14,887	7,015	6,657	9,500
	10^{-2}	15,506	21,933	15,906	15,250	6,330	7,566	8,906
	10^{-3}	14,036	22,145	11,957	13,127	5,756	7,531	8,857

* For explanation of antigens, see Methods section.

Table 3. Antibody specificity as percentage total IgG-αIgG binding in MSPRIA at 10^{-1} dilution

	Subject number						
Antigen	1	2	3	4	5	6	7
PA SON	65·3	58·4	45·8	12·6	1·0	1·9	3·0
PA LPS	29·4	48·6	74·5	0·2	2·2	1·2	1·5
SA	47·0	5·5	4·7	1·6	0	0·3	1·7
CA	1·7	25·2	15·1	0·7	0	1·0	5·2
AF	1·9	2·3	4·2	1·7	1·3	2·3	0·8
Trypsin	0·8	5·6	3·6	3·8	0·5	0	0
sIgA	14·9	7·1	2·2	1·8	2·3	0	5·1
Lavage	22·1	36·9	14·4	6·2	4·4	3·7	3·1

Affinity chromatography

The tubes corresponding to the Sephadex G150 macromolecular peak 1 (Fig. 1) were pooled, perevaporated, and applied to protein A-Sepharose. Neutral elution at pH 7·0 yielded a large protein peak containing IgM, and some IgG in CIC⊕ sera. Acid elution with 1 M acetic acid, pH 2·5, yielded a second distinct protein peak in CIC⊕ sera containing IgG and sometimes IgM, while CIC⊖ sera yielded little protein with no detectable immunoglobulin (Fig. 2).

Dissociation of isolated CIC

The acid eluates were each pooled, dialyzed, and then subjected to urea dissociation. They were then chromatographed on a second Sephadex G150 column. CIC⊖ sera had, as expected, no detectable protein or immunoglobulin in the Sephadex eluate. In contrast, CIC⊕ sera had two or more distinct protein peaks with IgG concentrated in fractions 75–90 (Fig. 3). These fractions were then tested for antibody specificity in the MSPRIA.

MSPRIA

Results of the MSPRIA are shown in Table 2. Patients 1–4 all showed positive titration curves for PA SON and/or LPS. The relative amount of PA IgG was estimated by determining the net counts min^{-1} of PA IgG mg^{-1} total IgG in serum and CIC. PA IgG in CIC was increased 102,9,18, and 22 times relative to serum values, respectively. Patient 1 also showed specificity for SA, while patients 2 and 3 reacted with CA. CF CIC⊕ subjects all showed higher binding to CF lavage fluid antigen(s) than CIC⊖ subjects, but this binding did not show typical antigen–antibody titration curves except for CF patient 2. To address the question of separation of IgG-CIC from monomeric IgG antibody, we studied CF patient 5, who was colonized with PA and had typical high CF PA IgG levels (Moss *et al.*, 1981), but who lacked CIC. This patient did not have any antibody to PA SON or LPS after this isolation–dissociation process. To address the question of disease-related specificity of the IgG-CIC, we studied pooled seropositive RA CIC⊕ sera. Again, no binding to any of the antigens employed was noted. In all runs, IgG was included in the MSPRIA as an antigen to estimate the total binding capacity of the system. Table 3 lists the antibody specificities found for each serum tested as a percentage of this total.

DISCUSSION

The use of sequential gel filtration and affinity chromatography on protein A-Sepharose for the isolation of CIC was initially described by Chenais *et al.* in 1977. Anti-RNA antibody was found in CIC from a patient with systemic lupus erythematosus and anti-IgG antibody was found in CIC from a patient with Sjögren's syndrome (Chenais *et al.*, 1977). These workers subsequently applied this technique to isolate anti-insulin antibody in CIC from a diabetic patient (Kilpatrick & Virella, 1980). Tucker *et al.* (1978) developed a similar methodology to isolate and characterize CIC in rats

bearing a Gross virus-induced lymphoma. In their report, they noted that some CIC formed *in vivo* with apparently high antigen–antibody affinity required urea dissociation after affinity chromatography for component identification (Tucker *et al.*, 1978). Herrman *et al.* (1978) also used urea to dissociate CIC, followed by gel filtration and subsequent identification of anti-basement membrane antibody in four patients with SLE. We combined these techniques with a highly sensitive microtitre radioimmunoassay (Moss & Lewiston, 1980) to identify a variety of antibody specificities in isolated CIC from patients with CF. The identification of antibacterial and antifungal antibodies in these CIC establish the pulmonary origin of CIC in CF and support prior evidence of endotoxin activity and antibody to pseudomonas lipopolysaccharide in CF CIC (Berdischewsky *et al.*, 1980). Antibody specificities also paralleled respiratory colonization data. We have previously associated the presence and levels of CIC in CF with exacerbations of chronic pulmonary disease (Moss *et al.*, 1981). Taken together, these findings suggest an immunopathogenetic mechanism for an undetermined portion of the progressive lung damage seen in CF.

We have also been interested in the possibility of autosensitization contributing to the immunopathology of CF. In 1964 Stein *et al.* reported that mucus but not serum from CF patients contained precipitins to CF but not normal lung tissue extract. Since then a variety of auto- or isoantibodies have been described in CF, including antibodies specific for reticulin fibre, colostrum, saliva, normal human serum, seminal fluid, nuclear antigens and allogeneic IgG (Wallwork *et al.*, 1974; Hoiby & Wiik, 1975). CIC from subject number 2 appeared to contain antibody activity to an unknown antigen or antigens in CF lung lavage.

The CIC in our CF patients did not contain IgG specific for normal human colostral secretory IgA (sIgA). Sensitization to an altered sIgA molecule, however, remains a possibility as alterations in CF sIgA have been reported (Wallwork & McFarlane, 1976). We have not detected precipitins to sIgA in CF serum, but we did find a significantly decreased prevalence of free secretory component in sol phase CF sputum when compared with controls with other respiratory diseases (unpublished data).

Because of pancreatic insufficiency, most CF patients receive oral pancreatin which contains xenogeneic trypsin. Work at our centre has established the immunogenic character of this therapy (Romeo *et al.*, 1978), and cross-sensitization to determinants on human trypsin (Denaro & Romeo,, 1980). We did not, however, find antibody specificity for trypsin in CIC from our CF patients.

In summary, we have found that microorganisms colonizing the respiratory tract, especially *Pseudomonas aeruginosa*, account for much of the antigenic specificity of antibodies present in CIC from patients with CF. This localizes the source of the CIC to the lungs and supports our prior association of CIC with lung disease in CF. We did not find evidence of antibodies to normal colostral secretory IgA or bovine trypsin in the CIC. Finally, other antigens in CF respiratory secretions, such as altered host glycoproteins, may also play a rôle in local immune complex formation.

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REFERENCES

ANH-TUAN, N. & NOVAK, E. (1981) Hepatitis B surface antigen circulating immune complexes (HBsAg-CICs) in patients with hepatitis B and asymptomatic HBsAg carriers. *Clin. exp. Immunol.* **43,** 246.

BERDISCHEWSKY, M., POLLACK, M., YOUNG, L.S., CHIA, D., OSHER, A.B. & BARNETT, E.V. (1980) Circulating immune complexes in cystic fibrosis. *Pediat. Res.* **14,** 830.

BJORVATN, B., BARNETSON, R.S., KRONVALL, G., ZUBLER, R.H. & LAMBERT, P.H. (1976) Immune complexes and complement hypercatabolism in patients with leprosy. *Clin. exp. Immunol.* **26,** 388.

CASALI, P. & LAMBERT, P.H. (1979) Purification of soluble immune complexes from serum using polymethylmetacrylate beads coated with conglutinin or C1q. *Clin. exp. Immunol.* **37,** 295.

CHENAIS, F., VIRELLA, G., PATRICK, C.C. & FUDENBERG, H.H. (1977) Isolation of soluble immune complexes by affinity chromatography using staphylococcal Protein-A-Sepharose as substrate. *J. Immunol. Meth.* **18,** 183.

DENARO, M. & ROMEO, G. (1980) Antibodies cross-reacting with porcine and human trypsin in cystic fibrosis. *Pediat. Res.* **14,** 1086.

GRAY, B.M. (1979) ELISA methodology for polysaccharide antigens. Protein coupling of polysaccharides for adsorption to plastic tubes. *J. Immunol. Meth.* **28,** 187.

GREENWOOD, F.C. & HUNTER, W.M. (1963) The preparation of ^{131}I-labeled human growth hormones of high specific radioactivity. *Biochem. J.* **89,** 114.

HAAKENSTAD, A.O. & MANNIK, M. (1977) The biology of immune complexes. In *Autoimmunity* (ed. by N. Talal), p 277. Academic Press, New York.

HANESSIAN, S., REGAN, W., WATSON, D. & HASKELL, T.H. (1971) Isolation and characterization of antigenic components of a new heptavalent *Pseudomonas* vaccine. *Nature: New Biology* **229,** 209.

HARKISS, G.D., BROWN, D.L. & EVANS, D.B. (1979) Longitudinal study of circulating immune complexes in a patient with *Staphylococcus albus*-induced shunt nephritis. *Clin. exp. Immunol.* **37,** 228.

HERRMANN, K., LOHRISCH, I., BÖHME, H.J. & HAUSTEIN, U.F. (1978) Detection of antibodies after immune complex splitting in serum of patients with bullous pemphigoid and systemic lupus erythematosus. *Br. J. Der.* **99,** 635.

HOIBY, N. & WIIK, A. (1975) Antibacterial precipitins and autoantibodies in serum from patients with cystic fibrosis. *Scand. J. Resp. Dis.* **56,** 38.

KILPATRICK, J.M. & VIRELLA, G. (1980) Isolation and characterization of soluble insulin-anti-insulin immune complexes formed *in vitro* and *in vivo* in serum from patients with diabetes mellitus. *Clin. exp. Immunol.* **40,** 445.

LAMBERT, P.H., DIXON, F.J., ZUBLER, R.H., AGNELLO, V., CAMBIASSO, C., CASALI, P., CLARKE, J., COWDERY, J.S., MCDUFFIE, F.C., HAY, F.C., MACLENNEN, I.C.M., MASSON, P., MULLER-EBERHARD, J.H., PENTINEN, K., SMITH, M., TAPPEINER, G., THEOFILOPOULOS, A.N. & VERROUST, P.A. (1978) A collaborative study for the evaluation of eighteen methods for detecting immune complexes in serum. *J. Lab. clin. Immunol.* **1,** 1.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.L. (1951) Protein measurement with Folin phenol reagent. *J. biol. Chem.* **193,** 265.

MCFARLANE, H., HOLZEL, A., BRENCHLEY, P., ALLEN, J.D., WALLWORK, J.C., SINGER, B.E. & WORSLEY, B. (1975) Immune complexes in cystic fibrosis. *Br. med. J.* **i,** 423.

MOSS, R.B. & LEWISTON, N.J. (1980) Immune complexes and humoral response to *Pseudomonas aeruginosa* in cystic fibrosis. *Am. Rev. Respir. Dis.* **121,** 23.

MOSS, R.B., HSU, Y.P. & LEWISTON, N.J. (1981) ^{125}I-C1q binding and specific antibodies as indicators of pulmonary disease activity in cystic fibrosis. *J. Pediatr.* **99,** 215.

PERNICE, W., SODOMANN, C.P., LÜBEN, G.L., SEILER, F.R. & SEDLACEK, H.H. (1979) Antigen-specific detection of HbsAg-containing immune complexes in the course of hepatitis B virus infection. *Clin. exp. Immunol.* **37,** 376.

ROMEO, G., PARSONS, M., BOSSEN, A., BLESSING-MOORE, J. & CAVALLI-SFORZA, L. (1978) Trypsin-binding IgG in cystic fibrosis. *Nature (London)* **274,** 909.

SCHIOTZ, P.O., HOIBY, N., JUHL, F., PERMIN, H., NIELSEN, H. & SVEHAG, S.-E. (1977) Immune complexes in cystic fibrosis. *Acta. pathol. microbiol. scand. C* **85,** 57.

SCHIOTZ, P.O., NIELSEN, H., HOIBY, N., GLICKMANN, G. & SVEHAG, S.-E. (1978) Immune complexes in the sputum of patients with cystic fibrosis suffering from chronic *Pseudomonas aeruginosa* lung infection. *Acta. pathol. microbiol. scand. C.* **86,** 37.

SCHIOTZ, P.O., SORENSEN, H. & HOIBY, N. (1979) Activated complement in the sputum from patients with cystic fibrosis. *Acta. pathol. microbiol. scand. C* **87,** 1.

STEIN, A.A., MANLAPOS, F.C., SOIKE, K.F. & PATTERSON, P.R. (1964) Specific isoantibodies in cystic fibrosis. *J. Pediatr.* **65,** 495.

TABBARAH, Z.A., WHEAT, L.J., KOHLER, R.B. & WHITE, A. (1980) Thermodissociation of staphylococcal immune complexes and detection of staphylococcal antigen in serum from patients with *Staphylococcus aureus* bacteremia. *J. clin. Microbiol.* **11,** 703.

THEOFILOPOULOS, A.N. & DIXON, F.J. (1979) The biology and detection of immune complexes. *Adv. Immun.* **28,** 89.

TUCKER, D.F., BEGENT, R.H.J. & HOGG, N.M. (1978) Characterization of immune complexes in serum by adsorption on staphylococcal Protein A. Model studies and application to sera of rats bearing a Gross virus-induced lymphoma. *J. Immunol.* **121,** 1644.

WALLWORK, J.C., BRENCHLEY, P., MCCARTHY, J., ALLAN, J.D., MOSS, A.D., MILFORD WARD, A., HOLZEL, A., WILLIAMS, R.F. & MCFARLANE, H. (1974) Some aspects of immunity in patients with cystic fibrosis. *Clin. exp. Immunol.* **18,** 303.

WALLWORK, J.C. & MCFARLANE, H. (1976) The SIgA system and hypersensitivity in cystic fibrosis. *Clin. Allergy* **6,** 349.

WOOD, R.E., BOAT, T.F. & DOERSHUK, C.F. (1976) Cystic fibrosis. *Am. Rev. Respir. Dis.* **113,** 833.

YOSHINOYA, S., COX, R.A. & POPE, R.M. (1980) Circulating immune complexes in coccidiomycosis. Detection and characterization. *J. clin. Invest.* **66,** 655.

ZUBLER, R.H. & LAMBERT, P.H. (1976) The ^{125}I-C1q binding test for the detection of soluble immune complexes. In *In Vitro Methods in Cell-Mediated and Tumor Immunity* (ed. by B.R. Bloom & J.R. David), p. 565. Academic Press, New York.

ZUBLER, R.H. & LAMBERT, P.H. (1977) Immune complexes in clinical investigation. In *Recent Advances in Clinical Immunology* (ed. by R.A. Thompson), vol. 1, p. 125. Churchill Livingstone, New York.