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Longitudinal study of circulating immune complexes in a patient with *Staphylococcus albus*-induced shunt nephritis

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SUMMARY

The direct measurement and partial characterization of circulating immune complexes has been performed in a longitudinal study of a patient with *Staphylococcus albus*-induced shunt nephritis. The high levels of immune complexes were associated with cryoglobulinaemia and hypocomplementaemia. The activation of complement was found to be via the classical pathway, but the functioning of the alternative pathway may have been impaired *in vivo* due to very low levels of C3. The host response to the infection was also characterized by the production of a marked macroglobulinaemia, high titres of rheumatoid factor and a typical acute phase increase in the C-reactive protein level.

Immune complex levels were persistently elevated many months after the removal of the focus of the infection. A possible explanation for this surprising finding may lie in the nature of the antigens in the immune complexes. It was found that the immune complexes contained both antibodies to and antigens from *Staphylococcus albus*. In particular, glycerol teichoic acid and staphylococcal nuclease were identified as components of the immune complexes present during the acute phase. Glycerol teichoic acid was also identified in the immune complexes found later although other *Staphylococcus albus* antigens as yet unidentified were also present and persisted in the circulation for several months.

INTRODUCTION

Shunt nephritis is a hypocomplementaemic glomerulonephritis associated with chronically infected ventriculoatrial shunts, where the microorganism is usually a coagulase-negative staphylococcus (Stickler *et al.*, 1968; Bolton *et al.*, 1975; Dobrin *et al.*, 1975; Strife *et al.*, 1976). It has been suggested that the renal damage associated with this syndrome is due to the deposition of soluble circulating immune complexes, since the glomerular lesions resemble those seen in experimentally induced immune complex disease and in systemic lupus erythematosus (Kaufman & McIntosh, 1971; Rames *et al.*, 1970; Strife *et al.*, 1976). However, no detailed studies of soluble immune complexes have been reported to date, and the association between shunt infection, circulating immune complexes and nephritis has remained conjectural.

With the advent of modern sensitive techniques for monitoring the levels of immune complexes in serum, a greater understanding of the pathogenesis of many human diseases has been achieved (Zubler & Lambert, 1977). In this report we describe the application of a new assay for measuring circulating immune complexes (Harkiss & Brown, 1979) to a serial study of a *Staphylococcus albus*-induced shunt nephritis. A detailed longitudinal analysis of the functional activities of complement components is also presented, along with measurements of other relevant immunological parameters. Finally, an attempt to identify the antigens and antibodies comprising the immune complexes is presented.

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MATERIALS AND METHODS

The patient's serum samples were stored in liquid nitrogen for complement studies and at −20°C for other investigations. Immune complexes were determined by the polyethyleneglycol-complement consumption test (PEG-CC test) (Harkiss & Brown, 1979) and by the ^{125}I-labelled C1q binding test (Zubler *et al.* 1976). In the PEG-CC test, immune complexes were first isolated from serum by precipitation in 2·5% PEG, then concentrated ten times. The redissolved complexes were assayed by measuring their ability to fix complement using a sensitive complement consumption procedure.

Total haemolytic complement (CH_{50}) was measured by a kinetic haemolytic assay (Lachmann & Hobart, 1973). C3 was measured immunochemically by rocket immunoelectrophoresis using sheep anti-human C3. The functional activities of C1, C2, C4, C6, C7, factor B and total haemolytic alternative pathway were measured as described by Lachmann & Hobart (1973). C3 breakdown products were determined by Laurell rocket immunoelectrophoresis. Ethylenediaminetetraacetic acid (EDTA) (Sigma) was added to fresh test sera, and the mixture electrophoresed for 3 hr at 12mA per plate. Electrophoresis was then continued in the second dimension into a gel containing sheep anti-human C3 antiserum at 6mA per plate for 16 hr. The plates were then examined for the presence of C3 breakdown products. Controls consisted of running similar mixtures of normal human serum (NHS) and EDTA.

C-reactive protein (CRP) and C1 esterase inhibitor were measured by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965), using antiserum to human CRP and human C1 esterase inhibitor obtained from Miles Laboratories Ltd., Slough. Rheumatoid factor was determined by agglutination of IgG-coated latex particles (Wellcome Reagents Ltd., Beckenham, Kent). Cryoglobulins were detected by incubating test sera at 4°C for 72 hr, then spinning at 1700 *g* for 10 min at 4°C. The isolated cryoglobulins were washed in cold phosphate buffered saline, and then redissolved in the latter at 37°C. Immunoglobulins and complement components present in the cryoglobulins were detected by Ouchterlony gel diffusion using sheep antisera. Renal deposits of immunoglobulins and complement components were detected by standard immunofluorescence techniques.

Antibodies to staphylococcal antigens were detected by Ouchterlony gel diffusion and counter-immunoelectrophoresis. Gel diffusion plates, used for the detection of antibodies and other components in cryoglobulins, were incubated at 37°C to maintain the solubility of the cryoglobulins. Immunoglobulin (Ig) fractions from sera were prepared by ammonium sulphate precipitation. The preparations were redissolved in one tenth of the original volume of serum, then dialysed against saline. Further purification was carried out using ion-exchange chromatography on diethylaminoethyl cellulose (DEAE) (Watman Biochemicals Ltd., Maidstone, Kent). The IgG fraction was pooled and concentrated by pressure dialysis. The retarded proteins, including IgM and IgA, were eluted off the column by a 0·01 M phosphate buffer pH 8 containing 0·3 M NaCl, dialysed and concentrated by pressure dialysis (denoted 'IgM fraction').

Glycerol teichoic acid was purified from *Staphylococcus albus* cell walls by treating them with trypsin, ribonuclease and pepsin, (Sigma Chemical Company) followed by extraction in 1 M HCl at 100°C for 20 min (Morse, 1963). The polysaccharide nature of this extract was confirmed by its reaction with concanavalin A (Sigma Chemical Company) (Reeder & Ekstedt, 1971). Micrococcal nuclease (grade VI) from *Staphylococcus aureus* (Foggi strain) was obtained from Sigma and was dissolved in saline at a concentration of 1·3 mg/ml.

Rabbits were immunized with three deep intramuscular injections, spaced successively 1 week and 10 days apart, of sonicated solutions containing equal volumes of PEG precipitates from the patient's serum and Freund's complete adjuvant. Blood samples were obtained 7–21 days after the injection period. A total of four rabbits were immunized, two with an initial preparation obtained in July 1977 (P1), and two with a preparation obtained 5 months later (P2).

Haematological and biochemical parameters were measured by standard techniques by the departments of Haematology and Biochemistry, Addenbrooke's Hospital, Cambridge. Blood, urine and cerebrospinal fluid (CSF) culture was performed using standard techniques by the Department of Bacteriology at Addenbrooke's Hospital.

CASE REPORT

The patient was a 34-year-old woman who had developed hydrocephalus and a right-sided spasticity, following a sub-arachnoid haemorrhage in 1970. A ventriculoatrial shunt was inserted and the patient remained well until early 1977. She then complained of gradual onset of tiredness, lethargy, nausea, anorexia and exertional dyspnoea. She had no history of chest pain or dysuria, but had developed ankle swelling 2 months prior to presentation. She had also noticed increasing thirst over this period. On examination, she was pale but not oedematous; she had normal heart sounds and her chest was clear. She was anaemic with a haemoglobulin level of 4·9 g/dl, had a erythrocyte sedimentation rate (ESR) of 95 mm/hr and a minor diffuse increase in gammaglobulins.

She was found to be in renal failure, with blood urea and creatinine values of 24·4 mmol/l and 577 μmol/l respectively. The 24 hr protein excretion was 5·8 g, while leucocytes, erythrocytes and casts were noted in the urine. *E. coli* sensitive to Ampicillin was grown from urine culture. Tests for the presence of antinuclear factors were negative and LE cells were not demonstrated. Liver function tests were normal

and chest X-ray revealed no lung disease or cardiac enlargement. The kidneys were found to be of normal size and position.

The suspected diagnosis of shunt nephritis was confirmed when a penicillin-resistant *Staph. albus* was grown first from blood cultures, and subsequently from the excised shunt. Blood cultures were consistently positive for *Staph. albus* until the shunt was removed in July 1977, and thereafter were negative. Culture of one sample of CSF in July was negative. The patient recovered steadily after removal of the shunt, though her renal function improved only very slowly and she remained in renal failure, with abnormal blood urea and creatinine values and persistent proteinuria 1 year after resolution of the infection.

A renal biopsy performed in July 1977 indicated the presence of a type 1 membranoproliferative glomerulonephritis. The light microscopy appearances showed enlarged glomeruli with mesangial and cellular proliferation, and there was thickening of the basement membrane capillary loops with marked narrowing of the lumen. A few glomeruli showed marked crescent formation. The basement membranes, examined by silver stains, appeared split. Immunofluorescence studies revealed a diffuse granular fluorescence of the peripheral capillary loops, with antiserum to IgG, IgM, C3 and C1q. Some granular and linear staining with fibrogen was evident. Electron microscopic examination revealed a scattered fusion of epithelial foot processes. Many capillary membranes were split with interposition of mesangial cell cytoplasm, but there was no definite evidence of electron dense deposits.

RESULTS

Serial profiles of immune complexes and other immunological parameters

The results of serial measurements of immune complexes and complement components are shown in Fig. 1. Very high levels of immune complexes were observed, while C1, C2, C4, C3 and total haemolytic complement measurements were markedly depressed. The shunt was removed and the bacteraemia stopped by a 1 week course of chloramphenicol. A lag of about 6 weeks was noted following removal of the shunt before immune complex levels dropped significantly. Furthermore, these did not fall within the normal range until January 1978, a lapse of several months. Similarly, complement components took several weeks, and in the case of C3 and C4, several months, before reaching their normal ranges.

Immune complexes measured by the ^{125}I-C1q binding test showed a very similar pattern, with a lag of several weeks before the levels dropped substantially. Cryoglobulins were also found during the month of July. These were of the mixed type containing IgG, IgM, C3 and C1q, and when added to fresh normal human serum were strongly anticomplementary. They disappeared from the serum as the patient recovered.

Measurements of the functional activity of factor B of the alternative pathway indicated little alteration in this parameter during the acute phase. However, measurement of the total haemolytic activity of the alternative pathway initially gave zero values, but became normal on resolution of the infection. Laurell analysis of C3, performed on sera obtained during the acute phase, indicated that almost all of this component was in the form of breakdown products. C6 and C7 were in the normal range throughout, as were C1 esterase inhibitor levels (not shown).

Analysis of immunoglobulins (Table 1) indicated that there was a marked increase in IgM levels which dropped slowly towards normal values, while IgA and IgG were slightly depressed and normal respectively. Rheumatoid factor was also noted to be present in high titres during the acute phase, disappearing gradually over a period of several months. In addition, a sharp increase in CRP concentrations was evident during July 1977, but these subsided relatively quickly after the removal of the shunt. The blood urea and creatinine profiles remained abnormal (not shown), and together with the persistence of proteinuria indicated continuing renal impairment with only a slow improvement in renal function during the period of study.

Characterization of antibodies and antigens present in the patient's serum

An attempt was made to characterize antibodies to *Staph. albus* to gain further insight into the

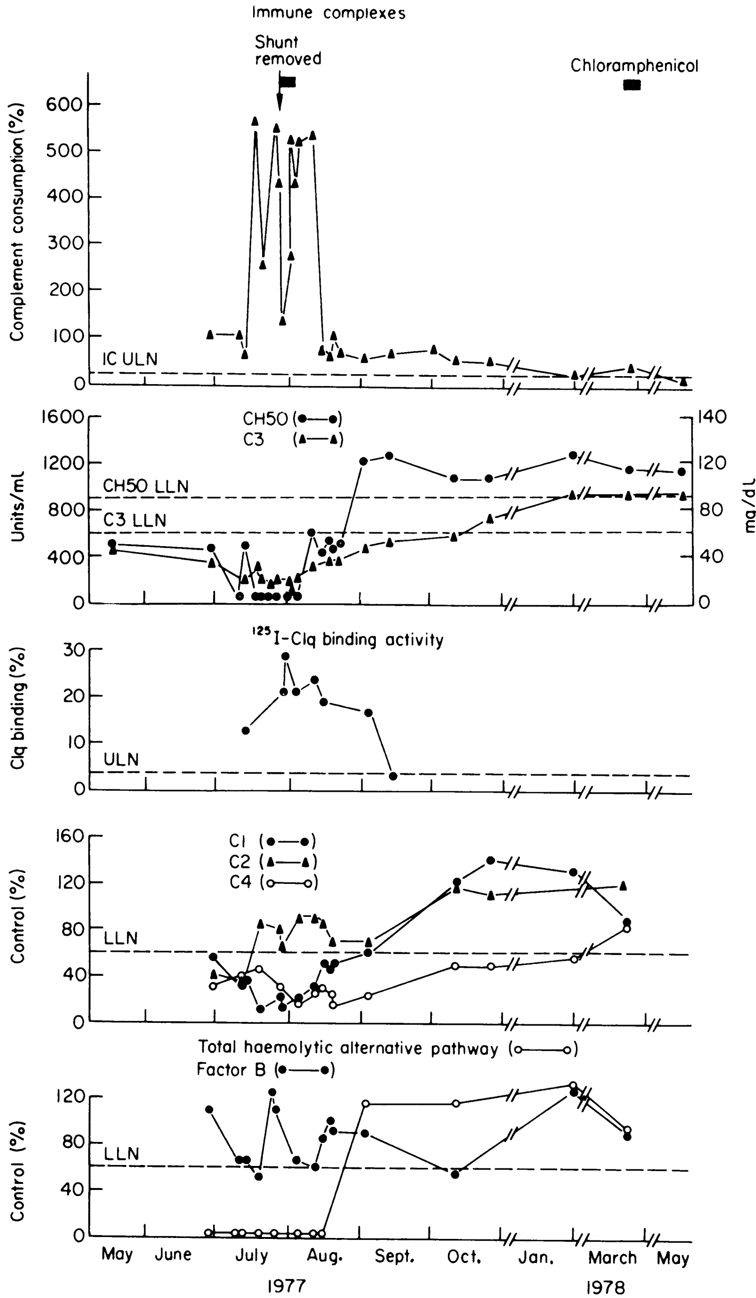

FIG. 1. Longitudinal measurements of immune complexes, total haemolytic complement, C1, C2, C3, C4, factor B and total haemolytic alternative pathway in a patient with *Staph. albus*-induced shunt nephritis. ULN and LLN are the upper and lower limits of normal, respectively.

composition of the immune complexes. The patient's sera were found to contain antibodies which reacted on counter-immunoelectrophoresis with a sonicated preparation of the *Staph. albus* cultured from the excised shunt. Precipitin lines were obtained on counter-immunoelectrophoresis of serial serum samples taken throughout the 12 month period following the bacteraemia (Table 1).

As indicated in Table 2, antibodies to *Staph. albus* antigens were also found to be present in the patient's serum by gel diffusion. In addition, the isolated cryoglobulins showed strong reactions with the

TABLE 1. Serial analysis of IgM, rheumatoid factor, C-reactive protein and antibody to *Stap. albus* in a patient with shunt nephritis

Date	IgM (g/l)	RF titre	CRP (μg/ml)	Antibody to *Staph. albus*
1977 July 13th	2·7	512	45	+
July 21st	4·1	1024	27	+
July 27th	7·0	256	86	+
Aug. 1st	5·6	2048	19	+
Aug. 11th	5·7	512	12	+
Sept. 2nd	3·1	256	22	+
Oct. 26th	1·8	128	12	+
Nov. 29th	1·4	64	13	+
1978 Mar. 23rd	—	4	—	+
Normal range	0·5–2·0	0–1/64	0–6·0	—

TABLE 2. Ouchterlony analysis of antibodies to the *Staph. albus* sonicate present in the patient's serum, cryoglobulin, IgG, IgM fraction and serum PEG precipitate, and present in the rabbit P1 and P2 antisera. Normal human and rabbit sera were run as controls

	Staph. albus sonicate	*Number of lines*
Patient J.W.:		
Serum	+	2
Cryoglobulin	++++	3
DEAE-purified IgG	++++	3
DEAE IgM fraction	++	2
PEG precipitate	++++	3
Rabbit anti-PEG ppt. (P1)	++++	3
Rabbit anti-PEG ppt. (P2)	++	2
Normal human serum	–	0
Normal rabbit serum	–	0

Staph. albus antigens. This observation suggested that the immune complexes in the form of cryoglobulins were in antibody excess. The antibody reactivity was found to reside in both DEAE-purified IgG and in the eluted IgM fraction. Artificial immune complexes were prepared *in vitro* using the IgG antibody and the soluble *Staph. albus* antigens. These immune complexes were found to be strongly anticomplementary when assayed in the PEG-CC test, indicating the presence of complement-fixing subclasses of IgG.

The initial stage of the PEG-CC assay (see the Materials and Methods section) involved precipitating and concentrating immune complexes in 2·5% PEG. This procedure was also used to prepare a suitable solution of the patient's immune complexes for injection into rabbits. The rationale for this was that any *Staph. albus* antigens present in the immune complexes would elicit a corresponding antibody response in the rabbits. This proved to be the case, and two rabbit antisera were raised which reacted with three *Staph. albus* antigens. The PEG precipitates used for immunization also contained antibodies directed against at least three bacterial antigens (Table 2).

When this procedure was repeated at the end of November 1977, several months after the blood cultures had become negative, a similar response was obtained with two fresh rabbits, but in this instance two precipitin lines were obtained on gel diffusion analysis (Table 2). An *in vitro* control experiment was carried out to determine if any *Staph. albus* antigens could be precipitated by 2·5% PEG. Various amounts of the concentrated *Staph. albus* preparation were added to normal human serum and PEG was

added to give a final concentration of 2·5%. The redissolved precipitates were tested on gel diffusion against the patient's IgG, and also against the P1 rabbit antisera. No reaction was evident, indicating that *Staph. albus* antigens were not present in the PEG precipitate.

When the patient's IgG and the rabbit antisera were reacted on the same plate against the *Staph. albus* sonicate, one of the precipitin lines obtained showed a reaction of identity (Fig. 2). This indicated that both the patient's and the rabbit's antibodies were directed against the same bacterial antigen, and thus further implicated the latter as a constituent of the patient's immune complexes.

The results of preliminary investigations with extracts from *Staph. albus* cell walls are shown in Table 3. Precipitating antibodies to glycerol teichoic acid were found in both the IgG and IgM fractions of the patient's serum (Fig. 2), while strong reactivity was found between both the P1 and P2 rabbit antisera and the glycerol teichoic acid using counter-immunoelectrophoresis (Fig. 3). The patient's immunoglobulin fraction also contained antibody activity against staphylococcal nuclease, but this was relatively weak. The P1, but not the P2, rabbit antisera gave strong reaction against the purified nuclease (Fig. 4).

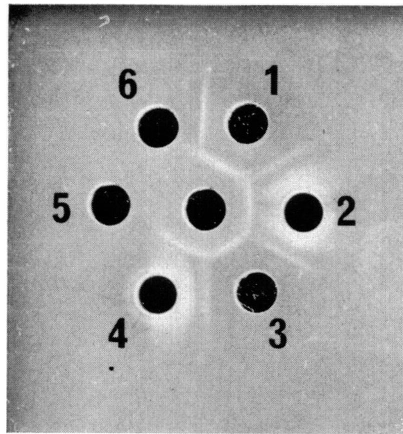

FIG. 2. Ouchterlony analysis showing the reaction between the *Staph. albus* sonicate and (a) the rabbit P1 Igs, (b) the control rabbit Ig pool, and (c) the patient's purified IgG. Also shown is the reaction of identity between the patient's purified IgG and the rabbit P1 Igs when run against the *Staph. albus* sonicate. The rabbit Igs show reactions against the patient's IgG. Peripheral wells contain: (1) patient's IgG; (2) P1 rabbit Igs: (3) patient's IgG; (4) P1 rabbit Igs; (5) normal rabbit Ig pool; (6) *Staph. albus* glycerol teichoic acid. Centre well contains the *Staph. albus* sonicate.

TABLE 3. Ouchterlony analysis of antibodies to *Staph. albus*-glycerol teichoic acid and to staphylococcal nuclease present in the patient's Ig's and in the rabbit P1 and P2 Igs. Normal human and rabbit Igs were run as controls

Antibody	Glycerol teichoic acid	Staphylococcal nuclease
JW Ig fraction	+++	+
JW IgG	+++	n.d.
JW IgM fraction	++	n.d.
Rabbit anti-PEG ppt. (P1)*	+++	++
Rabbit anti-PEG ppt. (P2)*	++	—
Normal human Ig fraction*	—	—
Normal rabbit pool Ig fraction*	—	—

* Whole immunoglobulins (Igs) concentrated by ammonium sulphate precipitation. n.d. = Not done.

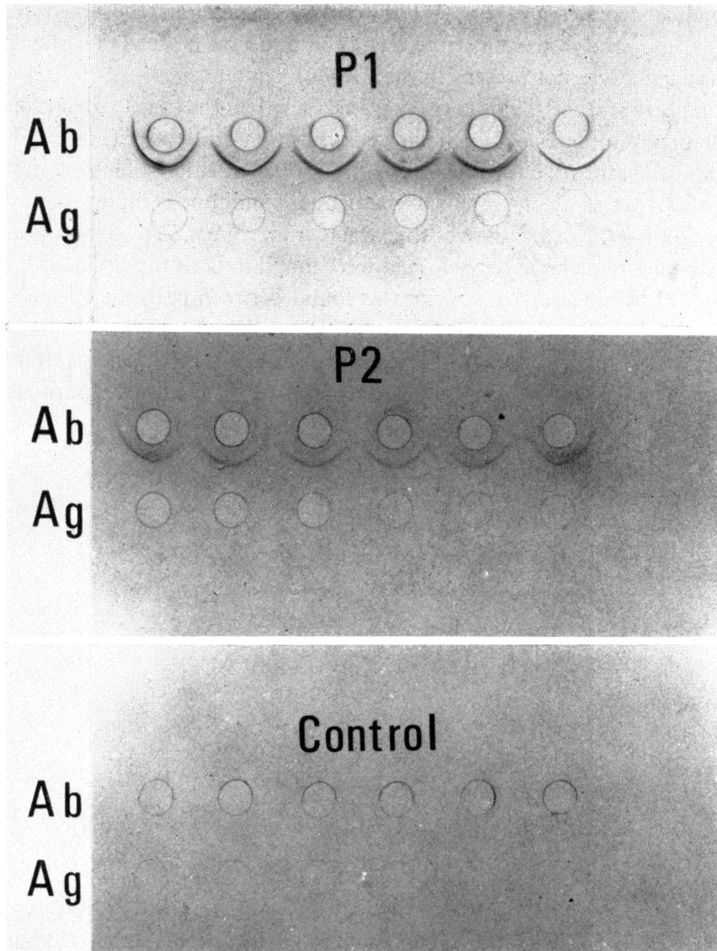

FIG. 3. Reaction between the rabbit P1 and P2 Igs and serial dilutions of purified *Staph. albus* glycerol teichoic acid by counterimmunoelectrophoresis. A normal rabbit Ig pool was run as a control. Ab and Ag are the antibody and antigen wells, respectively.

DISCUSSION

Shunt nephritis is now an established clinical syndrome. Though relatively rare (Schoenbaum, Gardner & Shillito, 1975), some aspects of its pathogenesis have been elucidated in recent years. The infecting organism is usually *Staphylococcus albus*, but *Staph. aureus*, *Listeria monocytogenes*, *Corynebacterium bovis*, *Bacillus subtilis*, *Bacillus cereus*, *alpha haemolytic streptococcus*, *micrococcus* and *Candida albicans* have also been grown (Stickler *et al.*, 1968; Holt, 1969; Moncrieff *et al.*, 1973; Bolton *et al.*, 1975; Dobrin *et al.*, 1975; Strife *et al.*, 1976; Schoenbaum *et al.*, 1975; Peeters *et al.*, 1978). There is a marked activation of complement via the classical pathway, usually in association with cryoglobulinaemia. Rheumatoid factor is frequently found in the serum and may also be found in the cryoglobulin, and the renal histology is similar to that of lupus nephritis, with subendothelial and mesangial deposits of IgG, IgM, C1q and C3 (Strife *et al.*, 1976). Bacterial antigens have also been demonstrated within the glomerulus (Kaufman & McIntosh, 1971; Dobrin *et al.*, 1975).

The observation that shunt infections are linked with the finding of renal deposits of immunoglobulins and complement has led to the belief that the renal damage is a consequence of the deposition of circulating immune complexes in the glomerulus (Moncrieff *et al.*, 1973; Strife *et al.*, 1976). There have,

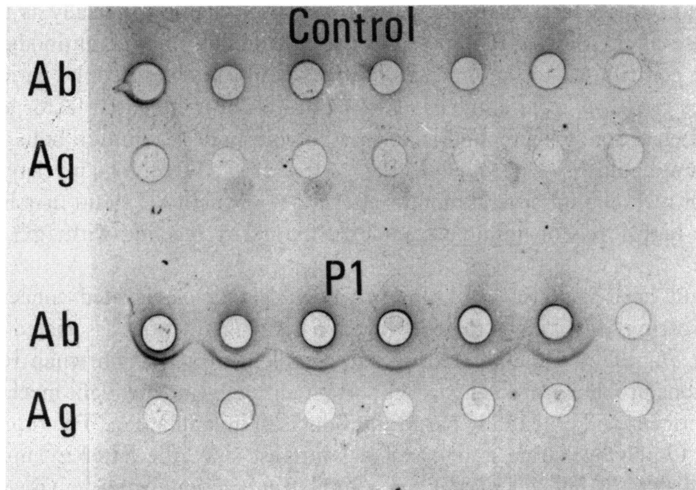

FIG. 4. Reaction between the P1 and P2 rabbit Igs and serial dilutions of purified staphylococcal nuclease by counterimmunoelectrophoresis. A normal rabbit Ig pool was run as a control. Ab and Ag are the antibody and antigen wells, respectively.

however, been no detailed studies of circulating immune complexes in shunt nephritis and to date no serial studies of immune complex levels have been published. In the present study, immune complexes measured by two methods were unequivocally found to be present in the serum of a patient with shunt nephritis. Very high levels of immune complexes were found during the acute phase of the illness, but these subsequently dropped to much lower values following removal of the shunt, which in this case was the focus of the infection. The observation that immune complex levels remained outside the normal range for over 6 months was remarkable and suggested either persistent antigenaemia or, more remotely, some form of autoimmune reaction.

Detailed analysis of functional levels of complement components in the classical pathway revealed an inverse relationship with the immune complex levels. The lag of 6 weeks before immune complex and complement levels started to return to normal is also noteworthy and unexplained, but may have reflected saturation of the reticuloendothelial system owing to the very high load of circulating complexes. Such a situation would have favoured increased deposition of immune complexes in the kidney (Haakenstad & Mannik, 1974) and may have contributed to the observed renal damage. Dobrin *et al.* (1975) noted a similar delay in one of their patients before haemolytic C3 and CH_{50} returned to normal. Functional levels of C1, C2 and C4 in their patient became normal after 10 days, whereas in our patient, C3 and C4 were persistently low. This is, of course, consistent with the continued presence of elevated levels of immune complexes.

Activation of the alternative pathway in shunt nephritis appears to occur less frequently than activation of the classical pathway, and when it does take place, it tends to be associated with more severe disease (Dobrin *et al.* 1975; Strife *et al.* 1976). Since factor B levels in our patient were essentially normal throughout the course of the illness, the finding of zero values for total haemolytic alternative pathway was unexpected. One explanation for this may relate to the observation that almost all of the C3 present in the test sera during the acute phase was in the form of breakdown products. Thus the lack of any functional C3 would have prevented activation of the alternative pathway, and would have artefactually produced a zero value on haemolytic assay. However a similar situation may have prevailed *in vitro*, preventing activation and subsequent recruitment of the alternative pathway and leaving the latter essentially intact. Such a situation could conceivably have contributed to the pathogenesis of the disease. It is known that both pneumococcal and staphylococcal teichoic acid fix complement via the alternative pathway (Winkelstein & Tomasz, 1978; Wilkinson *et al.*, 1978) and this may be one mechanism whereby such antigens are eliminated from the circulation.

The macroglobulinaemia observed in our patient was also notable, especially as the peak coincided with the acute phase of the illness. It is possible that production of IgM antibodies to the infecting organism may have contributed to the increased macroglobulin levels since the patient's IgM fraction contained antibodies to *Staph. albus* antigens. The high levels of rheumatoid factor may also have contributed to the IgM elevation. Rheumatoid factor has previously been found in both serum and isolated cryoglobulins in shunt nephritis (Dobrin *et al.*, 1975; Strife *et al.*, 1976). The persistently elevated titres of rheumatoid factor recorded for several months after the removal of the shunt may have contributed at least in part to the high levels of immune complexes found, by reacting with IgG bound in existing immune complexes.

Another important host response to chronic infection is that of elevated concentrations of CRP. This acute phase protein is now known to be involved in many interactions associated with inflammatory processes (Claus *et al.*, 1977). Its ability to initiate complement activation when reacting with polyanions and polycations probably represents an important antibody-independent mechanism of clearance of some bacterial antigens and potentially harmful products of inflammation. The reaction between CRP and pneumococcal C polysaccharide is of particular interest since the latter is known to be a ribitol teichoic acid (Brundish & Baddiley, 1968). Thus, a similar interaction may be envisaged *in vivo* between CRP and the teichoic acid of *Staph. albus*, enhancing the clearance of this antigen from the circulation.

An investigation into the nature of the antigens and antibodies comprising the immune complexes revealed the presence in the patient's serum of antibodies to at least three antigens released by sonication of the bacteria. Antibodies were found against both purified staphylococcal nuclease and glycerol teichoic acid released from the cell wall by acid extraction. In addition, the reaction between the P1 rabbit antisera and the sonicated preparation of *Staph. albus*, indicated the presence of bacterial antigens in the form of immune complexes in the patient's serum. Since the P1 antisera gave a strong reaction with glycerol teichoic acid on counter-immunoelectrophoresis, it would seem that the latter was a major antigen in the PEG precipitates used for immunization. In addition, the reaction between the P2 rabbit antisera and glycerol teichoic acid suggested that the persistent antigenaemia seen in the patient was due in part to this antigen. Strife *et al.* (1976), using intraperitoneal injections of cryoglobulins adsorbed onto bentonite particles, also produced strong antisera in rabbits to teichoic acid from *Staph. albus.*

The P2 rabbit antisera, however, reacted strongly with at least two antigens in the sonicated preparation of the bacteria, indicating that there was persistence in the patient's blood, 5 months after resolution of the bacteraemia, of other antigens from *Staph. albus* which continued to form immune complexes with circulating antibodies. The fact that the P2 preparation was obtained after several months of negative blood cultures ruled out the possibility of whole bacteria being present in the immunization mixture.

Smialowcz & Schwab (1977), in experiments with streptococcal cell walls, found that rat macrophages and human monocytes could not process the cell walls of certain strains, and that this resulted in the persistence of the antigens in the culture fluid. It is therefore possible that some cell wall antigens from *Staph. albus* may have persisted in the circulation for long periods due to 'treadmilling' by macrophages (Howard *et al.*, 1969). The binding and retention of antigens on macrophage surfaces is certainly known (Unanue, 1972). In addition, the release of antigens from lysosomal 'storage compartments' within macrophages by exocytosis and the persistence in serum and tissues of undigestible antigens, such as polysaccharides, for prolonged periods has been described (Weissmann & Dukor, 1970; Janeway & Humphrey, 1968; Felton *et al.*, 1955). It is therefore possible that these cells may act as a reservoir of antigen long after the resolution of an infection. This mechanism could explain the persistently elevated measurements of immune complexes and the slow recovery of renal function noted in our patient and in some patients reported by others (Dobrin *et al.*, 1975; Strife *et al.*, 1976).

Many antigens from *Staph. albus*, in addition to the cell wall glycerol teichoic acid, have been described in recent years. These include antigen D, agglutinogens, sensitizing antigens and several haemolysins (Oeding & Grov, 1972; Jeljaszewicz, 1972). Many enzymes such as hyaluronidase, staphylokinase, nuclease, phosphatase and lipase have also been recorded (Abramson, 1972; Wadström, 1974). Further studies are required to determine which, if any, of these antigens are nephritogenic in man, though Sakomoto (1968) has shown that the α-haemolysin of *Staph. albus* is nephritogenic in rabbits and may be

worthy of study in this context. In addition, the observation that teichoic acid produces an immunosuppressive effect when injected into mice (Ekstedt, 1974) suggests an important role for this antigen in determining the host's immune response to staphylococcal infection, and certainly merits future attention.

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REFERENCES

Abramson, C. (1972) Staphylococcal enzymes. *The Staphylococci* (ed. by J. O. Cohen), p. 187. Wiley-Interscience, New York.

Bolton, W.K., Sande, M.A., Normansell, D.E., Sturgill, B.C. & Westervelt, F.B., Jr (1975) Ventriculojugular shunt nephritis with *Corynebacterium bovis*. Successful therapy with antibiotics. *Am. J. Med.* **59,** 417.

Brundish, D.E. & Baddiley, J. (1968) Pneumococcal C-substance, a ribitol teichoic acid containing choline phosphate. *Biochem. J.* **110,** 573.

Claus, D.R., Siegel, J., Petras, K., Skor, D., Osmand, A.P. & Gewurz, H. (1977) Complement activation by interaction of polyanions and polycations. III. Complement activation by interaction of multiple polyanions and polycations in the presence of C-reactive protein. *J. Immunol.* **118,** 83.

Dobrin, R.S., Day, N.K., Quie, P.G., Moore, H.L., Vernier, R.L., Michael, A.F. & Fish, A.J. (1975) The role of complement, immunoglobulin and bacterial antigen in coagulase-negative staphylococcal shunt nephritis. *Am. J. Med.* **59,** 860.

Ekstedt, R.D. (1974) Immune response to surface antigens of *Staphylococcus aureus* and their role in resistance to staphylococcal disease. *Ann. N.Y. Acad. Sci.* **236,** 203.

Felton, L.D., Prescott, B., Kauffman, G. & Ottinger, B. (1955) Pneumococcal antigenic polysaccharide substances from animal tissues. *J. Immunol.* **74,** 205.

Haakenstad, A.O. & Mannick, M. (1974) Saturation of the reticuloendothelial system with soluble immune complexes. *J. Immunol.* **112,** 1939.

Harkiss, G.D. & Brown, D.L. (1979) Detection of immune complexes by a new assay, the polyethyleneglycol-complement consumption test (PEG-CC). *Clin. exp. Immunol.* **36,** 117.

Holt, R. (1969) The classification of staphylococci from colonized ventriculoatrial shunts. *J. clin. Path.* **22,** 475.

Howard, J.G., Elson, J., Christie, G.H. & Kinsky, R.G. (1969) Studies on immunological paralysis. II. The detection and significance of antibody-forming cells in the spleen during immunological paralysis with type III pneumococcal polysaccharide. *Clin. exp. Immunol.* **4,** 41.

Janeway, C.A., Jr. & Humphrey, J.H. (1968) Synthetic antigens composed exclusively of L-or -D amino acids. II. Effect of optical configuration on the metabolism and fate of synthetic polypeptide antigens in mice. *Immunology*, **14,** 225.

Jeljaszewicz, J. (1972) Toxins (Haemolysins). *The Staphylococci* (ed. by J.O. Cohen), p. 249. Wiley-Interscience, New York.

Kaufman, D.B. & McIntosh, R. (1971) The pathogenesis of the renal lesion in a patient with streptococcal disease, infected ventriculoatrial shunt, cryoglobulinaemia and nephritis. *Am. J. Med.* **50,** 262.

Lachmann, P.J. & Hobart, M. (1973) Complement Technology. *Handbook of Experimental Immunology* 2nd edn (ed. by D. M. Weir). Blackwell, Oxford.

Mancini, G., Carbonara, A.O. & Heremans, J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2,** 235.

Moncrieff, M.W., Glasgow, E.F., Arthur, L.J.H. & Hargreaves, H.M. (1973) Glomerulonephritis associated with *Staphylococcus albus* in a Spitz–Holter valve. *Arch. Dis. Child.* **48,** 69.

Morse, S.I. (1963) Isolation and properties of a group antigen of *Staphylococcus albus*. *J. exp. Med.* **117,** 19.

Oeding, P. & Grov, A. (1972) Cellular antigens. *The Staphylococci* (ed. by J.O. Cohen), Wiley-Interscience, New York.

Peeters, W., Mussche, M., Becaus, I. & Ringoir, S. (1978) Shunt nephritis. *Clin. Nephrol.* **9,** 122.

Rames, L., Wise, B., Goodman, J.R. & Piel, C.F. (1970) Renal disease with *Staphylococcus albus* bacteraemia. A complication in vetriculoatrial shunts. *JAMA*, **212,** 1671.

Reeder, W.J. & Ekstedt, R.D. (1971) Study of the interaction of concanavalin A with staphylococcal teichoic acids. *J. Immunol.* **106,** 334.

Sakamoto, T. (1968) Studies on the infectious allergy of staphylococci experimental renal lesions caused by the sensitization with haemolytic staphylococci. *Jap. J. Alleg.* **17,** 357.

Schoenbaum, S.C., Gardner, P. & Shillito, J. (1975) Infections of cerebrospinal fluid shunts: epidemiology, clinical manifestations, and therapy. *J. Infect. Dis.* **131,** 543.

Smialowcz, R.J. & Schwab, J.H. (1977) Processing of streptococcal cell walls by rat macrophages and human monocytes in vitro. *Infect. Immunity* **17,** 591.

Stickler, G.B., Shin, M.H., Burke, E.C., Holley, K.E., Miller, R.H. & Segar, W.E. (1968) Diffuse glomerulonephritis associated with infected ventriculoatrial shunt. *N. Engl. J. Med.* **279,** 1077.

Strife C.F., McDonald, B.M., Ruley, E.J., McAdams, A.J. & West, C.D. (1976) Shunt nephritis: The nature of the serum cryoglobulins and their relation to the complement profile. *J. Pediatr.* **88,** 403.

Unanue, E.R. (1972) The regulatory role of macrophages in antigenic stimulation. *Adv. Immunol.* **15,** 95.

Wadström, J. (1974) Biological properties of extracellular proteins from staphylococcus. *Ann. N.Y. Acad. Sci.* **236,** 343.

Weissman, G. & Dukor, P. (1970) The role of lysosomes in immune responses. *Adv. Immunol.* **12,** 283.

WINKELSTEIN, J.A. & TOMASZ, A. (1978) Activation of the alternative complement pathway by pneumococcal cell wall teichoic acid. *J. Immunol.* **120**, 174.

WILKINSON, B.J., KIM, Y., PETERSON, P.K., QUIE, P.G. & MICHAEL, A.F. (1978) Activation of complement by cell surface components of *Staphylococcus aureus*. *Infect. Immunity*, **20**, 388.

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

ZUBLER, R.H., LANGE, G., LAMBERT, P.H. & MIESCHER, P.A. (1976) Detection of immune complexes in unheated sera by a modified ^{125}I-C1q binding test. Effect of heating on the binding of C1q by immune complexes and application of the test to systemic lupus erythematosus. *J. Immunol.* **116**, 232.