ANTIBODY IN THE TREATMENT OF IMMUNE COMPLEX DISEASES

BY

RIAD-NOUR EL-DIN-EL-ASFARI

A Thesis submitted for the Degree of
Doctor of Philosophy
in the University of London

July 1981

Department of Experimental Pathology
St. Mary's Hospital Medical School
Paddington
London W2 1PG

This thesis is dedicated to
the fondest memory of my
father and to my mother
and my wife

ABSTRACT

This thesis is a study of the effects of plasma or gamma globulin transfusion of patients with either immune complex disease or pericarditis/myocarditis. Remission occurred in some disease groups for prolonged periods.

The basic observation made before I started this work was that some patients with immune complex disease, particularly with systemic lupus erythematosus would respond to transfusion with plasma or gamma globulin.

I have studied circulating complexes of the patients to see if the response to transfusion from a particular donor could be predicted from a study of the cross-reaction between the donor antibodies in the plasma and the patient's antigens in the circulating complexes. Masson in Belgium has suggested such a correlation in a small series which he carried out.

The method for detecting cross-reactions did not show a clear correlation with results of transfusion. Study of the complexes did, however, show that many patients apparently had a deficient immune response to their presumed foreign antigens. Some patients only make IgM antibody against the complexed antigen without IgG, whereas the unaffected members of the family or blood donors do make IgG antibody. The results of intra-familial transfusion have been studied.

It is possible that the patients do not have T cells of the required specificity and are unable to generate cytotoxic T cells or make co-operative IgG antibody. Without these killing of virus infected cells by cytotoxic T cells or K cells and IgG antibody is not possible. Transfusion of IgG provides a mechanism for K cell killing of virus infected cells with consequent remission of the disease.

Measuring antigenic cross-reaction by radioimmunoassay has provided evidence for common causal agents in a group of patients by showing antigenic cross-reaction between their immune complexes. This may help in the future to identify the antigens which are responsible for the disease and to find a proper therapy to eliminate them.

ACKNOWLEDGEMENTS

The work described in this thesis was carried out between April 1978 and July 1981 in the Department of Experimental Pathology at St. Mary's Hospital Medical School.

I wish to express my sincere thanks and appreciation to my supervisor Professor James F. Mowbray, for giving me the opportunity to work in his department, and for his continual encouragement and helpful guidance throughout the study.

I am very grateful to Mrs. Jill Burton-Kee for her technical assistance.

I would like to thank everyone in the Department for their help and encouragement and especially Mrs. D. Zewdie and Mr.S.G. Johannes who kindly permitted me to cite certain of their experimental data.

Finally, I am indebted to the University of Aleppo, Syria, for a scholarship to pursue my postgraduate studies.

CONTENTS

CONTENTS (contd.)

LIST OF TABLES

LIST OF TABLES (contd.)

LIST OF TABLE (contd.)

LIST OF FIGURES

CHAPTER ONE
INTRODUCTION

Glomerulonephritis

Classification of glomerulonephritis

A. Histological classification

1. Acute diffuse proliferative glomerulonephritis.
2. Rapidly progressive proliferative glomerulonephritis (sub-acute glomerulonephritis, Crescentic glomerulonephritis).
3. Mesangial prolferative glomerulonephritis.
4. Mesangio-capillary glomerulonephritis (Membrane-proliferative glomerulonephritis).
5. Focal proliferative glomerulonephritis.
6. Membranous glomerulonephritis.
7. Minimal change glomerulonephritis.
8. Focal sclerosis glomerulonephritis.

B. Classification of immunopathogenetic mechanisms.

I. Diseases known to result from immunological mechanisms.

a. Immune complex glomerulonephritis. This condition results from the deposition of circulating antigen-antibody complexes (immune complexes) in the glomerulus. This mechanism is responsible for the majority of immunologically mediated renal diseases in man, such as post-streptococcal glomerulonephritis, shunt nephritis and lupus nephritis.

b. Antiglomerular basement membrane disease. In this condition the antibodies are directed towards the glomerular basement membrane and cause the renal injury. This mechanism is responsible for a minority of renal disease, i.e. Good-Pasture's disease.

II. Diseases without evidence of immunological mechanisms.

In this condition neither immune complex nor antiglomerular basement membrane antibody appear to play a role in the pathogenesis of the disease such as minimal lesion glomerulonephritis. Recent evidence (Levinsky et al, 1978) supports the old findings (Ngu et al, 1970) that there might be a causal relationship between the activity of the disease and the presence of complexes.

Glomerulonephritis in experimental animals

A. Immune complex disease

Serum sickness

There are two types of experimental serum sickness.

Acute type

This type of lesion is usually produced when a group of experimental animals (usually rabbits) receive a single intravenous injection of bovine serum albumin (BSA)(250mg per kg body weight or more). After injection a number of rabbits develop inflammatory lesions in the hearts, joints, arteries and kidneys (Germuth, 1953; Hawn and Janeway, 1947).

Following the injection of BSA, its concentration in the plasma falls rapidly over the first 48 hours as it diffuses through the intravascular and extracellular fluids, then the plasma concentration of BSA falls more gradually as it is slowly metabolized, and disappears from the circulation at the end of 11 to 13 days; at this time free anti-BSA antibody is detectable in the serum (Wilson and Dixon, 1972; Germuth, 1953; Germuth and Rodriguez, 1973). Anti-BSA antibody appears in the circulation on about the seventh day after the initial injection of BSA (Wilson and Dixon, 1972; Dixon, 1971). This antibody binds to the circulating antigen to form the circulating immune complexes which are small in size at the beginning because the antigen predominates in great excess; these small immune complexes cannot fix complement and the renal lesion does not develop. As more antibody becomes available and when the circulating antibody and antigen are near equivalence, the immune complexes enlarge and can fix complement (Wilson and Dixon, 1972; Unanue and Dixon, 1967; Dixon, 1971).

These immune complexes disappear from the circulation on the eleventh day, by phagocytosis (Wilson and Dixon, 1972). Some of these complexes are trapped in the glomeruli, become attached to their capillary walls

and cause the renal lesion. The nephritis is usually characterized by proteinuria, cylindruria and an elevated blood urea (Germuth and Rodriguez, 1973; Unanue and Dixon, 1967; Dixon, 1971).

The glomerular changes were observed in over 80% of Germuth's (1953) animals which were killed on 12th and 15th days after the injection. By light microscopy the glomerulonephritis of serum sickness is characterized by proliferation of mesangial and endothelial cells. A small number of neutrophils may be present (Germuth, 1953; Germuth and Rodriguez, 1973; Hawn and Janeway, 1947).

By electron microscopy deposits of electron dense material blended into the basement membrane can be seen (Feldman, 1958). A similar form of glomerulonephritis can be produced in experimental animals by intravenous injection of prepared soluble immune complexes (Benacerraf et al, 1960).

Acute serum sickness is a self-limiting condition in which recovery starts as soon as all circulating antigen is eliminated (Hawn and Janeway, 1947).

Chronic type

In this type, the experimental animals (rabbits) received a daily intravenous injection of foreign protein over a long period of time (Dixon et al, 1961). These rabbits are divided into 3 main groups according to their antibody production (Unanue and Dixon, 1967; Dixon et al, 1961; Germuth and Rodriguez, 1973). The first includes animals that produce a large amount of antibody. They may develop glomerular lesions similar to those in acute serum sickness (Unanue and Dixon, 1967; Dixon et al, 1961). The rabbits of the second group do not form antibody to the foreign protein and glomerular injury does not occur. The third group includes rabbits that form an amount of antibody sufficient to result in the formation of circulating immune complexes in a moderate antigen excess. These complexes are usually detected

for some hours after each antigen injection (Unanue and Dixon, 1967; Dixon et al, 1961; Wilson and Dixon, 1972). In these animals chronic membranous and/or proliferative glomerulonephritis usually develop (Germuth and Rodriguez, 1963; Dixon, 1971). This lesion is usually progressive and fatal.

Other forms of experimental immune complex GN

1. Autologous immune complex nephritis (Heymann nephritis)

In this experimental type, animals are injected repeatedly with homologous tubular or renal tissue in Freund's adjuvant. Chronic progressive membranous glomerulonephritis usually develops and is manifested by severe proteinuria (Heymann et al, 1959; Edgington et al, 1968). The renal lesion is due to the formation of antibody-antigen complexes which deposit in the glomeruli. It has been demonstrated by Edgington et al (1967-1968) that the antigen which is responsible for the immunological stimulus is derived from the brush border of the proximal convoluted tubule.

2. Viral infection induced glomerulonephritis

Chronic viral infections (induced or natural) in animals can cause immune complex glomerulonephritis in which the antigens are of viral origin (Dixon et al, 1969). This type of glomerulonephritis has been established in animals with persistent lymphocytic choriomeningitis (LCM) virus, gross leukaemia virus, equine infectious anaemia virus and lactic dehydrogenase virus (Dixon et al, 1969; Wilson and Dixon, 1972; Hotchin and Collins, 1964; Mellors et al, 1971).

3. Immune complex disease in New Zealand mice

Mice of this strain have been found spontaneously to develop SLE-like illness, splenomegaly, haemolytic anaemia, and antinuclear antibody and rheumatoid factor in their serum. Some of the mice also develop

chronic glomerulonephritis (Dixon et al, 1969; East et al, 1967).

At least two antibodies can be eluted from the kidneys of the mouse

a. Antibodies against nuclear antigens (Dixon et al, 1969).

b. Antibodies directed against antigens of the virus which infects this animal naturally (Mellors et al, 1971; Dixon et al, 1969).

B. Antiglomerular basement membrane disease (Nephrotoxic serum nephritis)

This type of renal injury is produced by antibodies directed towards the glomerular basement membrane, and characterized by the presence of smooth linear deposition of IgG along the glomerular capillary walls (Germuth and Rodriguez, 1973; Unanue and Dixon, 1967). There are two types of experimental antiglomerular basement membrane disease.

1. Heterologous antiglomerular basement membrane antibody or passive antibasement membrane glomerulonephritis

In this experimental model the animal, e.g. rabbit, is injected with preparations of renal tissue or glomerular membrane obtained from an animal of a different species, e.g. rat. The rabbit then develops antibodies to the rat glomerular basement membrane. Serum from this rabbit containing circulating antibodies to rat kidney, is isolated and injected into the animal from which the renal tissue was obtained (rat)(Germuth and Rodriguez, 1973).

The nephritis occurs in 2 phases: (1) The primary or heterologous phase; this may occur within a few hours of the injection, when the heterologous nephrotoxic antibodies are attached to the glomerular basement membrane of the rat. The glomerulonephritis which occurs in this phase is usually transient. The heterologous immunoglobulin can be demonstrated by immunofluorescence along the glomerular basement membrane in linear pattern (Germuth and Rodriguez, 1973; Unanue and Dixon, 1967). (2) The secondary or autologous phase; this appears

5 to 7 days after the injection when the host (rat) develops an antibody response to the heterologous nephrotoxic antibodies and is characterized by reaction of the host (rat) antibodies with the heterologous antibasement membrane antibodies which remain attached to the glomerular basement membrane. In this phase, severe glomerulonephritis usually develops (Unanue and Dixon, 1965; Germuth and Rodriguez, 1973).

2. Autologous antiglomerular basement membrane antibody or active antibasement membrane glomerulonephritis

This type can be produced in the animals by injection of heterologous or homologous glomerular basement membrane preparations in Freund's adjuvant. This causes the animals to form antibodies which react with their own glomerular basement membrane and provoke the renal injury, which is usually severe and fatal (Germuth and Rodriguez, 1973; Wilson and Dixon, 1972).

Human glomerulonephritis

Acute proliferative glomerulonephritis

In this disease the renal injury is most probably due to the deposition of immune complexes within the glomeruli, similar to those occurring in acute serum sickness; streptococcal infection is the major cause (James, 1972). Post-streptococcal glomerulonephritis is discussed.

Acute post-streptococcal glomerulonephritis (APSGN)

This type of glomerulonephritis occurs most often in children, but also affects adults at any age (Leaf and Cotran, 1980). It often follows infection with group A, β-haemolytic streptococci type 12 (Wilmers et al, 1954) and type 4 in the majority of cases (Leaf and Cotran, 1980), but other types are occasionally involved, like type 49 (red lake)(Kaplan et al, 1970), type 1 (Goldsmith et al, 1958) and type 55 (Potter et al, 1968). There is a latent period of 1 to 2 weeks between the antecedent streptococcal infection and the development of nephritis and sometimes more than one month, with an average of 14 days (de Wardener, 1973; Schwartz and Kassirer, 1971).

Clinical immunology

One or more antibodies to the extracellular antigens or the haemolytic streptococcus are frequently identified in the serum during the development of acute GN, such as antistreptolysin (ASO), antistreptokinase (ASK), antihyaluronidase (AH) and antideoxyribonuclease (ADNase). An elevated titre of any of these antibodies is reliable evidence of a recent streptococcal infection (Taranta and Uhr, 1971). ASO titre is the most widely used serologic test, because it is easily titrated and more likely to increase after a streptococcal infection. It is raised in the serum about 20 days after the initial infection in 70-80% of patients (Schwartz and Kassirer, 1971; Kilbourne and Loge, 1948).

These types of antibodies give little or no immunity to the patient infected with these organisms (Schwartz and Kassirer, 1971; Black, 1970).

In patients treated with penicillin at the beginning of the infection, the frequency of an ASO response is reduced from the usual 70-80% to between 10-15% (Kassirer and Schwartz, 1961; Kilbourne and Loge, 1948). Antibodies form in response not only to the extracellular antigen of the streptococcus but also to the M protein, the cellular antigen, which gives streptococcus its type specificity. These antibodies give prolonged immunity that follows streptococcal infections, antibodies to the M protein develop slowly as late as 2 months after the infection (Kassirer and Schwartz, 1961) and may remain demonstrable for many years (Black, 1970). The antigens involved in this type of nephritis are most likely to be products of the infecting organism itself; but the evidence of the presence of the streptococcal components within the glomeruli is still controversial. Treser et al (1970), using fluorescein-labelled antisera to purified streptococcal membranes, stated that the streptococcal antigen was a part of the membrane structure of the streptococcal cell. Zabriskie (1971) failed to localize streptococcal membrane antigens in those patients who exhibited positive staining with type 12 antiserum prepared against the whole streptococcal organisms. Feldman et al (1966) failed to demonstrate any streptococcal antigens in any of their 9 patients. The reason may be that these antigens are presumably present in the circulation for short periods of time and the antigenic sites in the glomerular deposits would quickly be covered up by the excess of antibody (Zabriskie, 1971).

Clinical presentation

Gross or microscopic haematuria is usually present in the majority of patients and the urine is often described as "smoky". Microscopic haematuria was present in all patients studied by Kaplan et al (1970)

and Anthony et al (1967) and in 71% of those studied by Poon-King et al (1967). Haematuria is usually accompanied by red cell casts. Oedema is found in the majority of patients and is usually generalised. Physical examination revealed generalised oedema in all of the 681 patients of Poon-King et al (1967), while only 40% of the patients of Kaplan et al (1970) had a history of oedema. Proteinuria, is usually less than 3g, and massive proteinuria and nephrotic syndrome are relatively uncommon (James, 1972; Leaf and Cotran, 1980). Proteinuria was present in 40% of Kaplan et al (1970) patients and in 98% of Poon-King et al (1967) patients, while Crofton and Truelove et al (1948) reported a case of acute GN without albuminuria. Mild to moderate hypertension is usual and subsides with the diuresis or with the improvement of the renal function (James, 1972). Oliguria may occur but it is usually of short duration (Schwartz and Kassirer, 1971).

Pathology

Light microscopy

Usually, all glomeruli are affected and show hypercellularity due to diffuse proliferation of the mesangial and endothelial cells. This proliferation is accompanied by infiltration with polymorphonuclear leukocytes. There is an increased amount of mesangial matrix (Feldman et al, 1966; Burkholder, 1974). The capillary basement membrane usually shows no thickening and remains thin but occasionally may be focally thickened. The epithelial cells are not usually involved in the proliferative process and crescents are not common and seen only in severe cases (Heptinstall, 1974; Burkholder, 1974). Protein materials, red blood cells, casts and polymorphonuclear leukocytes are often present within the tubules. Hyaline or granular casts are less frequent. In the interstitium there may be some degree of oedema. Arteries and arterioles rarely show changes (Heptinstall, 1974).

Electron microscopy

The presence of electron dense granular deposits in the sub-epithelial space is the most prominent abnormality in this disease. These deposits, referred to as "humps" (Germuth and Rodriguez, 1973; Heptinstall, 1974), are usually observed at the early stages of the disease and disappear after 6 weeks (Cameron, 1979; de Wardener, 1973; Heptinstall, 1974), epithelial cell foot processes may show focal areas of fusion. Sub-endothelial and mesangial matrix deposits are sometimes seen. Swelling of the endothelial and mesangial cells and occasional discontinuities in the basement membrane are other electron microscopic findings (Andres et al, 1978; Leaf and Cotran, 1980).

Immunofluorescence

IgG and/or C3 are often present in granular patterns along the glomerular basement membrane (Heptinstall, 1974; Andres et al, 1978; Germuth and Rodriguez, 1973). The staining of C3 usually appears more intense and more constant than that of immunoglobulin.(Heptinstall, 1974), but Feldman et al (1966) reported that staining of C3 was less intense than that of immunoglobulin. IgG and C3 may be seen in the mesangial region. Deposits of properdin have also been reported to be present in the glomeruli in APSGN (Westberg et al, 1971). Streptococcal antigens have been identified in some cases in the early oourse of the disease (Treser et al, 1970; Zabriskie, 1971).

Serological findings

Serum complement concentrations are reduced during the acute phase of the disease, but usually return to normal within 4 to 8 weeks (Cameron, 1979; Leibowitch et al, 1980; Rodriguez-Iturbe et al (1980). Circulating immune complexes may be detected during the acute illness (Mohammed et al, 1977; Rodriguez-Iturbe et al (1980).

Prognosis

The prognosis of APSGN in children is usually good and the majority recover. In a series by McCrory et al (1959) no deaths were reported and 34 of the 35 children he studied were completely healed two years after the onset and only one had an abnormal Addis count. The course in adults is less favourable, some of them develop persistent GN and others die from other complications. In follow-up studies in eleven adult patients of Kushner et al (1961) with acute GN seven were clinically healed and four had apparent clinical evidence of chronicity. Irreversible renal damage has resulted in 50% of Baldwin's (1977) patients in a long period follow-up and the better prognosis was in children.

Rapidly progressive glomerulonephritis (RPGN)(Crescentic GN, Subacute GN)

This type of proliferative glomerulonephritis is characterized by relentless activity of the renal lesion with rapid decline in the renal function resulting in death due to irreversible renal failure and uraemia within weeks or months. The essential histological feature is the presence of epithelial crescents in the majority of the glomeruli (Arieff and Pinggera, 1972; Leaf and Cotran, 1980). This syndrome may occur as an idiopathic entity or due to a variety of conditions which include acute post-streptococcal glomerulonephritis, Henoch-Schönlein purpura, systemic lupus erythematosus, polyarteritis nodosa and Goodpasture's syndrome (Heptinstall, 1974; Arieff and Pinggera, 1972; Stilmant et al, 1979; Cameron, 1979). The pathogenic mechanism of the renal injury may be mediated either by immune complex or anti-GBM antibody in some, or by unknown mechanisms in others (Glassock, 1979).

Clinical presentation

The commonest presentation is an abrupt onset of oliguria or anuria (Cameron, 1979), other patients may present with insidious onset of peripheral oedema (Arieff and Pinggera, 1972). Microscopic haematuria and proteinuria are common. Hypertension may or may not occur (Leaf and Cotran, 1980; Stilmant et al, 1979). This syndrome is more common in adults (Stilmant et al, 1979).

Pathology

Light microscopy

The most important pathological finding in RPGN is crescent formation in more than 50% of all glomeruli (Andres et al, 1978). It is believed that the crescents are formed by a proliferation of the parietal epithelial cells. This proliferation may be due to the reaction of the epithelial cells to the blood components such as fibrin and red blood corpuscles

which are exuded into the capsular space (Heptinstall, 1974; Andres et al, 1978). Atkins et al (1976) found that macrophages rather than epithelial cells were the major cell type in the crescent formation. Post-streptococcal glomerulonephritis must be suspected if the gomerular changes are uniform and if there is a moderate to marked proliferation of mesangial cells (Andres et al, 1978; Heptinstall, 1974). Adhesions between the glomerular capillary tufts and the capsular crescents are common (Burkholder, 1974). Epithelial proliferation and hyaline droplet degeneration are present in the proximal convoluted tubule. Casts and RBC are also present in the tubular lumen. Interstitial oedema is common (Heptinstall, 1974; Andres et al, 1978).

Electron microscopy

Sub-endothelial deposits may be found in some instances (Andres et al, 1978; Heptinstall, 1974), while in others no deposits can be found (Stilmant et al, 1979). When sub-epithelial deposits are found post-streptococcal GN must be suspected. Hypertrophy of mesangial cells and an increase in mesangial matrix may be seen, foot processes of the epithelial cells are usually absent and epithelial pseudo-villi may be present (Burkholder, 1974).

Immunofluorescence

The findings by immunofluorescence fall into 3 groups:

1. A granular pattern of IgG and C3 along the glomerular capillary walls can be found (this may denote post-streptococcal GN). IgM may be present (Burkholder, 1974; Andres et al, 1978).
2. Diffuse linear pattern of IgG alone or with C3 may be seen along the GBM, this occurs in anti-GBM disease (Arieff and Pinggera, 1972; Burkholder, 1974; Andres et al, 1978).
3. In this group no immunoglobulin can be demonstrated in the glomeruli (Stilmant et al, 1979; Arieff and Pinggera, 1972; Andres et al, 1978).

Serological findings

In anti-GBM antibody-mediated RPGN, circulating anti-GBM antibody may be found. Serum complement components may be normal or fall with the development of end-stage renal failure, and circulating immune complexes are not found (Glassock, 1979). If the lesion is due to immune complex deposition, serum complement levels may be depressed and circulating immune complexes may be found (Glassock, 1979).

Prognosis

The prognosis is usually poor for this group of patients; end-stage renal failure usually develops within weeks or months (Stilmant et al, 1979; Andres et al, 1978; Leaf and Cotran, 1980).

Mesangial proliferative glomerulonephritis

The histological changes seen in this disease are similar to those which occur in the healing phase of acute post-streptococcal glomerulonephritis, but the evidence of streptococcal infection is absent in the majority of cases, and the aetiology is still uncertain (Cameron, 1979; Migone et al, 1980).

Clinical presentation:

Patients present with recurrent haematuria and proteinuria (Cameron, 1979; White et al, 1970). A few patients may present with the nephrotic syndrome (Migone et al, 1980).

Pathology

Light microscopy

There is an increase in the mesangial matrix with or without an increase in mesangial cellularity (Drummond and Andres, 1978). Polymorph infiltration is usually absent or scanty. The capillary wall is usually of normal thickness although localized thickening may occur. Epithelial crescent formation and capsular adherence are not usually seen (White et al, 1970; Churg et al, 1970).

Electron microscopy

There is an increase in the mesangial matrix with or without an increase in the number of mesangial cells. The glomerular basement membrane is usually normal. Electron dense deposits are not usually present (Burkholder, 1974).

Immunofluorescence

It is either negative or shows a granular pattern of IgG with or without C3 deposition along the capillary wall of the lobule or in the mesangium (Burkholder, 1974). IgM and IgA may be present (Migone et al, 1980).

Serological findings

Serum C3 levels are usually normal in patients with mesangial PGN (White et al, 1970). Circulating immune complexes have been detected in some cases (Woodroffe et al, 1977).

Prognosis

The prognosis of this type of glomerulonephritis is usually good and the patients do well, although some patients may develop renal impairment (Cameron, 1979). White et al (1970) reported no deaths from their eight patients.

Mesangiocapillary glomerulonephritis (MCGN)
(Membrano-proliferative glomerulonephritis)

This condition is called mesangiocapillary glomerulonephritis because it is characterized by a diffuse increase in cellularity of the mesangium and an irregular thickening of the GBM, a number of patients with this disease show hypocomplementaemia. The early description of MCGN appeared when West et al (1965) described a group of children with chronic glomerular disease and persistent hypocomplementaemia.

Aetiology and pathogenesis

The aetiology of this disease is unknown and the antigens are not recognized. Persistent hypocomplementaemia is found in the majority of patients with MCGN.

Three main explanations of the persistent hypocomplementaemia have been considered:

1. Diminished synthesis of C3
2. Urinary loss
3. Increased consumption of C3

1. Diminished synthesis of C3

Ogg et al (1968) and Cameron et al (1970) found no evidence of reduced complement synthesized in patients with MCGN; while Colten et al (1973) provided evidence of impaired synthesis of C3. They proved that the liver tissue obtained from those patients could not synthesize C3 *in vitro* but was capable of synthesizing C2 and C5.

2. Urinary loss

This concept has not found general acceptance and the following observations rule out urinary loss as an important cause. Northway et al (1969) reported persistent low serum C3 levels in 2 patients with MCGN whose urine was free of protein. Cameron et al (1970) reported 6 cases with persistent hypocomplementaemia who were anuric

and who were maintained on regular dialysis.

3. Increased consumption of C3

It is believed in this hypothesis that persistent hypocomplementaemia is due to the presence of the circulating factor, the so-called C3 nephritic factor (C3 NeF), in the serum of patients with MCGN. It is able to activate the complement system via the alternative pathway whilst the classical pathway complement components (C1, C2, C4) stay normal (Spitzer et al, 1969; Valotta et al, 1971). However Ooi et al (1976) suggested that both pathways were activated in this disease.

Studies of the biochemical nature of C3 NeF have produced different results. Thompson (1972) found that patients with hypocomplementaemic MCGN had low serum IgG levels and high IgG3 levels when compared with normal subjects, patients with SLE or patients with other types of chronic renal diseases; indicating that C3 NeF is of immunoglobulin nature, but Vallota et al (1974) found that C3 NeF which was isolated from patients with hypocomplementaemic chronic glomerulonephritis was a protein distinct from immunoglobulins.

Complement deficiency and C3 NeF have been reported in patients with partial lipodystrophy (PLD) with MCGN (Peters et al, 1973; Mery et al, 1978), and without renal disease or before the development of the renal disease (Thompson and White, 1973; Sissons et al, 1976). It appears that the PLD antedated the development of nephritis.

Clinical presentation

This disease seems to be more common in older children and adolescents (Cameron, 1979). In some series, one-third of the patients presented with acute nephritic syndromes and the other two-thirds presented with proteinuria, haematuria and oedema (Cameron et al, 1970; Michael et al, 1971). In some other series more than 65% of patients presented with nephrotic syndrome (Habib et al, 1973; Magil et al, 1979). Hypertension

may occur; Habib et al (1973) reported the presence of hypertension in 25% of their child patients, Magil et al (1979) reported the presence of hypertension in 35% of their adult patients and in 48% of their children. Proteinuria is of non-selective type; in the majority of cases (Michael et al, 1971).

Pathology

Studies of the glomerular morphology of patients with MCGN have shown 2 main types:

Type I is characterized by the presence of electron dense deposits in the sub-endothelial space (Habib et al, 1973; Andres et al, 1978).

Type II (also called dense deposit disease) is characterized by dense deposits in the glomerular BM (Habib et al, 1973; Andres et al, 1978).

In both types there are huge mesangial deposits (Andres et al, 1978). See below.

Type I

Light microscopy

All glomeruli are usually affected and there is thickening of the capillary walls with proliferation of the mesangial cells and increase in the mesangial matrix, although the endothelial cells may also be increased. A double layer of basement membrane (tram track appearance) may be seen if the section is stained with PAS or PA-silver-methenamine and slight to moderate neutrophil accumulation can be seen in the glomerular tuft. A few epithelial crescents may be present in some instances (Andres et al, 1978; Habib et al, 1973; Davis et al, 1978) or absent in others (Magil et al, 1979). The blood vessels usually appear normal unless hypertension develops. The interstitial tissue may contain chronic inflammatory cells or may be normal (Heptinstall, 1974).

Electron microscopy

Granular dense deposits are usually seen on the endothelial side of the BM and in the mesangial area, hump-like deposits may be present (Habib et al, 1973; Heptinstall, 1974; Magil et al, 1979), or absent (Davis et al, 1978).

The intervention of mesangial cytoplasm between the glomerular basement membrane and the endothelial cell wall gives new basement membrane-like material (Davis et al, 1978; Magil et al, 1979; Heptinstall, 1974; Andres et al, 1978).

Immunofluorescence

Granular deposition of C3 along the glomerular basement membrane is the most constant finding and it can be seen in the mesangial region. Immunoglobulins are also found, usually IgG and IgM (Heptinstall, 1974; Davis et al, 1978; Habib et al, 1973). Properdin is also seen (Westberg et al, 1971).

Type II (Dense deposit glomerulonephritis)

Light microscopy

There is diffuse thickening of glomerular BM, all glomeruli are enlarged and show mesangial hypercellularity, which may be mild or moderate (Davis et al, 1978).

Neutrophil accumulation may be found within glomeruli. The epithelial crescents are usually more frequent than in Type I (Droz et al, 1977). When the deposits are increased in size a ribbon-like appearance can be seen if the section is stained by haematoxylin and eosin (H and E)(Morel-Maroger, 1976; Habib et al, 1973).

Electron microscopy

Electron microscopy is important for the diagnosis of this disease. It shows homogeneous dense deposits within the BM of the glomeruli,

within Bowman's capsule, and in the tubular basement membrane (Habib et al, 1973; Davis et al, 1978; Droz et al, 1977). Humps may be present (Habib et al, 1973; Davis et al, 1978). The deposits may be present in the mesangial matrix (Davis et al, 1978). Mesangial cell proliferation and an increase in the mesangial matrix are frequent but usually mild in the majority of cases (Habib et al, 1973; Davis et al, 1978; Droz et al, 1977).

Immunofluorescence

C3 is usually present in all cases, along both glomerular and tubular BM in granular pattern, mesangial deposits of C3 are usually found (Habib et al, 1973; Droz et al, 1977; Davis et al, 1978).

Immunoglobulins are usually absent (Heptinstall, 1974; Droz et al, 1977), but in some patients IgM and, to a lesser extent, IgG or IgA may be present (Habib et al, 1973; Davis et al, 1978).

Differential diagnosis between Type I & II

1. In the glomeruli C3 appears to be more irregular in type I than in type II where it is more homogeneous.

2. Immunoglobulins are more likely to be present in the glomeruli in type I.

3. Sub-endothelial and mesangial electron-dense deposits are observed in type I while dense deposits are seen within the GBM in type II (Andres et al, 1978).

Serological findings

Serum complement levels are reduced in the majority of patients with MCGN. C3 levels are decreased in the course of the disease in over 75% of patients and to a greater degree in type II than in type I (Ooi et al, 1976; Habib et al, 1973; Davis et al, 1978). C1q, C4 and C1 may be decreased in type I (Ooi et al, 1976). The early acting components are usually normal in type II (Leibowitch et al, 1980). C3 NeF is often found in MCGN, in particular in type II (Spitzer et al, 1969;

Ooi et al, 1976). Circulating immune complexes may be found in both types (Ooi et al, 1977).

Prognosis

The course of both forms is one of continuing disease, and spontaneous resolution is unusual; renal function may remain normal for several years before the chronic renal failure develops (Cameron, 1979). Chronic renal failure is highest in type II (Habib et al, 1973).

Focal proliferative glomerulonephritis (FPGN)

The histological changes which occur in this disease affect segments of some glomeruli while other glomeruli remain normal. This lesion may either occur as an isolated renal condition and is called Berger's disease or IgA nephropathy, or it is associated with systemic diseases such as, SLE, sub-acute bacterial endocarditis, Henoch-Schönlein purpura and Polyartritis nodosa which are discussed later in this chapter. Berger's disease is discussed.

Berger's disease (IgA Nephropathy or IgG-IgA Nephropathy)

This disease was described by Berger (1969), who observed IgA-C3 in the mesangium of 55 patients who had no systemic disease or history of acute glomerulonephritis.

The pathogenesis of this disease is not well defined, Roy et al (1973) suggested that the association of recurrent haematuria with respiratory infection and IgA deposition might suggest a causal relationship with mucosal infections. Berthoux et al (1978) reported that 40% of their patients with mesangial IgA-glomerulonephritis were HLA B35 and they believed that this fact raised the possibility of genetic linkage between HLA and the disease; while Brettle et al (1978) did not agree with these findings. They found HLA B35 in 18% of their patients with IgA-C3 nephropathy and in 13% of their control group. This disease is more common in males than females (3:2) in their teens and twenties (Yokoska et al, 1978; Ueda et al, 1977).

The patients usually present with repeated attacks of macroscopic or microscopic haematuria, which may follow upper respiratory tract infection and in some cases exertion (Davies et al, 1973). Mild proteinuria may be present and is usually less than 1 g/day (Yokoska et al, 1978; Davies et al, 1973; van der Peet et al, 1977). Hypertension is not common in children but it may accompany the adult form (Kapoor et

al, 1980; Berger, 1969).

Pathology

Light microscopy

The glomeruli show mild focal and segmental mesangial cell proliferation with increased mesangial matrix. The glomerular basement membrane is usually normal (van der Peet et al, 1977; Davies et al, 1973; Roy et al, 1973). Generalized and diffuse proliferation may be seen (Yokoska et al, 1978; van de Putte et al, 1974), and glomerular scarring or hyalinization may be observed. Van der Peet et al (1977) reported glomerular scarring or hyalinization in 14 patients and tubular degeneration and interstitial fibrinosis in 13 of 27 adult patients with IgA nephropathy.

Electron microscopy

The electron microscopy shows an increase in the mesangial cells and electron-dense deposits in the mesangium (van der Peet et al, 1977). Sub-endothelial deposits may also be found (van de Putte et al, 1974; Davies et al, 1973).

Immunofluorescence

IgA deposition can be seen in the mesangial area of the glomeruli. This is usually accompanied by C3 and IgG. IgM may also be seen (Davies et al, 1973; Roy et al, 1973; van der Peet et al, 1977).

Serological findings

Serum IgA levels may be elevated (van der Peet et al, 1977). Complement components are usually normal (Glassock, 1979). Circulating immune complexes may be detected by a variety of assays (Abrass et al, 1980; Woodroffe et al, 1977).

Prognosis

The course of the disease is benign and self-limiting in the majority of child patients (Berger, 1969; van de Putte et al, 1974). In the minority, the disease persists into adult life and the patients develop hypertension and renal failure. The course of the disease is less favourable in adults and the elderly (van der Peet et al, 1977; Kappor et al, 1980).

Idiopathic membranous glomerulonephritis (MGN)

This lesion is characterized by regular diffuse thickening of the capillary walls of the glomeruli. Some authors prefer to use the term idiopathic MGN when the aetiologic factors are not known (Heptinstall, 1974). This is to exclude other causes which exhibit thickening in the glomerular capillary walls such as SLE, malaria, syphilis and Hbs Ag.

It is primarily a disease of middle age but it also occurs in children, some cases have been diagnosed before the age of 2 years (Habib et al, 1973); it occurs more often in males (Gärtner et al, 1974; Pierides et al, 1977).

Pathogenesis

This condition is probably an immune complex disease which is similar to those seen in chronic experimental serum sickness, although other mechanisms have been suggested.

Evans (1974) suggested that human MGN might result from in situ formation of the complexes in the capillary walls of the glomeruli. Cameron (1979) and Gelfand et al (1975,1976) suggested that C3b receptors, which were demonstrated in the glomeruli, might play a role in the initiation of the disease.

Clinical presentation

Proteinuria with the nephrotic syndrome is present in more than 75% of cases whilst the rest present with non-nephrotic proteinuria (Habib et al, 1973; Row et al, 1975; Pierides et al, 1977). Proteinuria is usually non-selective (Cameron, 1979). Mild microscopic haematuria is present in 70-80% of cases (Gärtner et al, 1974; Habib et al, 1973). Hypertension may be found in adult patients; it was present in 64% of Pierides et al (1977) patients and in 61% of Gärtner et al (1974) patients,

but rarely found in Habib's patients, who were all children. Oedema, lipiduria and hypercholestraemia may occur.

Pathology

Light microscopy

The capillary walls of the glomeruli show diffuse uniform thickening. In early cases this change may be undetectable by light microscopy (Andres et al, 1978). As the disease progresses the thickening of the capillary walls increases and spiky-like projections appear which extend toward the epithelial cells. These spikes can be seen when the section is stained with periodic acid-methenamine (Heptinstall, 1974; Gluck et al, 1973).

The epithelial cells swell without crescent formation (Andres et al, 1978; Burkholder, 1974). Proliferation is usually absent (Churg et al, 1970) but occasionally a slight increase in mesangial cells may occur. Obsolescence of many glomeruli occurs at the end-stage of the disease (Gluck et al, 1973). Hyaline granules, doubly refractile lipid and casts can be observed in the cell of the proximal convoluted tubule in the early stage of the disease (Heptinstall, 1974; Gluck et al, 1973).

Electron microscopy

The most important finding in this disease is the presence of sub-epithelial electron-dense deposits. The electron microscopic findings during the course of the disease can be divided into 4 stages:

Stage I. This stage is characterized by generalized fusion of the foot processes and rare small electron-dense deposits which may be seen in the sub-epithelial area (Germuth and Rodriguez, 1973).

Stage II. In this stage the electron-dense deposits are large in size, more numerous and form a continuous sub-epithelial layer interrupted by newly formed spikes (Germuth and Rodriguez, 1973; Andres et al, 1978).

Stage III. In this stage membranous transformations are increased and the capillary loop is largely thickened. The dense deposits are pale

and less dense (Germuth and Rodriguez, 1973; Andres et al, 1978).

Stage IV. This stage represents the terminal stage of the disease. Sclerotic glomeruli can be seen and large masses of electron-dense deposits are usually present in the matrix (Germuth and Rodriguez, 1973; Andres et al, 1978).

Immunofluorescence

Diffuse granular deposits of IgG and C3 can be seen along the glomerular capillary wall with or without IgM (Row et al, 1975; Burkholder, 1974).

Serological findings

Haemolytic complement and C3 levels are usually normal (Glassock, 1979). Circulating immune complexes may be found (Pussell et al, 1978; Abrass et al, 1980).

Prognosis

The disease is usually progressive and it may take several years before significant renal failure occurs. Remission of the proteinuria usually occurs from time to time (Gluck et al, 1973). The course of the disease seems to be more benign in children than in adults (Habib et al, 1973).

Minimal lesion glomerulonephritis (MLGN) - Lipoid nephrosis

This disease is most common in children under the age of 5 years. The peak incidence is between 2 and 4 years of age (Cameron, 1979) but it can occur at any age, even in the elderly (Fawcett et al, 1971).

Aetiology and pathogenesis

The aetiology and pathogenesis of this disease are obscure and there is no evidence of immunological mechanism, either by immune complexes or antiglomerular basement membrane antibody, but the following observations and hypotheses may suggest an immunological role in this disease:

1. Shalhoub (1974) has suggested that the thymus plays a role in this disease, and a disorder of T cell function leads to the production of lymphokines which are toxic to the glomerular BM . He has supported his hypothesis by the following observations.

   a. Remission induced by measles
   b. Susceptibility of the patients to infection
   c. Remission by steroids and cyclophosphamide.
   d. Hodgkin's disease can cause changes similar to those observed in minimal changes glomerulonephritis.

2. Eyres et al (1976) reported that lymphocytes isolated from patients with MLGN had greater lymphocyte toxicity on renal epithelial cells (in vitro) than those obtained from normal or the proliferative glomerulonephritis group.

3. Gerber and Paronetto (1971) demonstrated IgE along the capillary walls of the glomeruli in patients with MLGN.

4. Levinsky et al (1978) reported elevated levels of circulating immune complexes in children with MLGN.

5. Ngu et al (1970) reported elevated levels of immunoconglutinin in children with MLGN.

6. Thomson et al (1976) reported that the frequency of HLA B12 is greater in children with minimal change than in adult controls, and atopic symptoms (hay fever, elevated serum IgE and positive prick test for grass pollen antigen), are more common in those with HLA B12.
7. Duffy (1969) observed myxovirus-like particles in renal glomerular epithelial cells and he believed that this might be the causative agent in this disease.

Clinical presentation

This group of patients usually presents with a nephrotic syndrome. In some patients there is a previous history of allergy (Thomson et al, 1976). Proteinuria is more than 3g/24 hour and is usually highly selective in children, but less selective in adults (Cameron et al, 1974). Hypoalbuminaemia, less than 2.5 g/100ml, is usual. Oedema occurs and is usually accompanied by a puffy face in children. Haematuria is never macroscopic and is usually not persistent. Hypertension is uncommon in this disease, but hypercholesteraemia and hypertriglyceridaemia usually occur (Cameron, 1979; Cameron et al, 1974).

Pathology

Light microscopy

The absence of any conspicuous abnormality in light microscopy is an essential feature of this disease. The glomeruli are usually normal and there is no cellular proliferation (Heptinstall, 1974; Cameron et al, 1974), but Churg et al (1970) observed a slight increase of the mesangial matrix in some biopsies. If the disease persists for a long time, mild thickening of the capillary wall may be seen (Heptinstall, 1974; Drummond and Andres, 1978). Tubules and interstitium are usually normal, with the exception of fatty and hyaline droplets in the proximal convoluted tubule (PCT) (Heptinstall, 1974; Burkholder, 1974).

Electron microscopy

The major change seen by electron microscopy is the fusion of the foot processes of the epithelial cells (Burkholder, 1974; Heptinstall, 1974), which is due to flattening and swelling of these processes. A layer of epithelial cytoplasm replaces the foot processes. These changes return to normal in remission (Leaf and Cotran, 1980). Fluid droplets can be seen in the endothelial cytoplasm and a number of vacuoles are also seen in the tubule cells (Burkholder, 1974; Heptinstall, 1974). Usually there are no electron-dense deposits here, but Duffy et al (1970) reported the presence of subendothelial deposits in 9 patients with lipoid nephrosis.

Immunofluorescence

Immunoglobulin and complement are absent from the glomeruli of the patients with MLGN. Gerber and Paronetto (1971) observed IgE in 7 patients with MLGN in the glomerular capillary walls in 'comma-like' pattern with the absence of IgG, IgM, IgA and complement. The presence of IgE has not been confirmed by Lewis et al (1973).

Serological findings

Elevated levels of circulating immune complexes have been detected in some patients with MLGN (Levinsky et al, 1978; Abrass et al, 1980). Serum complement components are usually normal (Ruddy et al, 1975; Mallick, 1977; Leaf and Cotran, 1980). There may be an abnormal decrease of serum IgG and increase in IgM levels (Giangiacomo et al, 1975).

Prognosis

Patients with minimal change glomerulonephritis (MLGN) have an excellent prognosis and over 90% exhibit a good response to corticosteroid treatment, and temporary or permanent remissions occur (Drummond and Andres, 1978).

Focal sclerosis glomerulonephritis

This idiopathic lesion is usually progressive and non-proliferative It is characterized by the presence of glomerular sclerosis in both focal and segmental distribution. This is usually accompanied by hyalinosis. The condition may be found at any age (Hyman and Burkholder, 1973), but it has been most frequently described in children and adolescents (Hyman and Burkholder, 1974; Newman et al, 1976; Nagi et al, 1971).

Clinical presentation

Some of the patients may present with nephrotic syndrome and others may present with asymptomatic proteinuria (Nagi et al, 1971; Jenis et al, 1974). This may be accompanied by haematuria. Hypertension may or may not be present (Newman et al, 1976; Hyman and Burkholder, 1973).

Pathology

Light microscopy

In the early stage of the disease the lesion is minor and there is mild mesangial matrix expansion which may be diagnosed as minimal change glomerulonephritis (Hyman and Burkholder, 1974; Nagi et al, 1971).

When the disease progresses some glomeruli show diffuse or segmental sclerosis with adhesion to Bowman's capsule, and contain hyaline deposits. Foam cells containing lipid can be seen in the area. Other glomeruli appear normal (Jenis et al, 1974; Hyman and Burkholder, 1973 and 1974).

Cellular proliferation is generally absent, but a slight increase in the mesangial area of the sclerosed glomeruli may be seen (White et al, 1970). Focal tubular atrophy can be seen, which may be accompanied by interstitial fibrosis (Jenis et al, 1974; Nagi et al, 1971).

Electron microscopy

The basement membrane in the affected area usually shows irregular thickening. Segmental fusion of the epithelial cell foot processes

can be seen in all glomeruli. This is accompanied by epithelial pseudo-villous formation (Newman et al, 1976; Hyman and Burkholder, 1973). Granular electron-dense deposits are observed in the subendothelial and paramesangial areas (Newman et al, 1976; Hyman and Burkholder, 1973 and 1974), although Nagi et al (1971) failed to demonstrate any dense deposits in their patients.

Spherical viral-like particles were demonstrated in 6 out of 16 patients of Jenis et al (1974).

Immunofluorescence

Granular segmental deposits of IgM and C3 are usually present in the sclerotic areas of the affected glomeruli, and to a lesser extent IgG and IgA (Newman et al, 1976; Jenis et al, 1974; Hyman and Burkholder, 1973 and 1974). Nagi et al (1971) failed to demonstrate any immunoglobulin or fibrin in their 7 patients with this disease.

Serological findings

Complement component levels are usually normal (Hyman and Burkholder, 1973; Jenis et al, 1974; Ruddy et al, 1975). Circulating immune complexes may be found in some patients (Woodroffe et al, 1977).

Prognosis

The disease is usually progressive and the prognosis is poor. Renal failure develops within a few years of the onset and the lesion is usually steroid resistant (Nagi et al, 1971; Jenis et al, 1974; Hyman and Burkholder, 1973).

Systemic lupus erythematosus and the kidney

Systemic lupus erythematosus is another example of immune complex disease (Kohler, 1973), which occurs mainly in females and affects many systems of the body (Hughes, 1975).

The aetiology of the disease remains elusive, indications of a genetic role in the aetiology of systemic lupus erythematosus have come from family and twin studies. Arnett and Shulman (1976) reported the presence of systemic lupus erythematosus in more than one member of the family. Block and Christian (1975) reported that the disease could be found in approximately 1-2% of the first degree relatives of SLE patients. Some authors consider that SLE has an infective aetiology. Gyorkey et al (1969) stated that a virus might be the agent producing the initial injury which provokes the immune mechanism. Panem et al (1976) isolated C-type viral antigens from renal biopsies of patients with lupus nephropathy and in 1978 they detected immune complexes containing HEL-12 virus antigens in kidneys of 43 out of 44 patients with systemic lupus erythematosus. Evans et al (1971) detected raised antibody titres to Epstein-Barr virus in 62 of the sera from 100 cases of SLE. Sunlight and drugs (such as hydrallazine and procainamide are other iniating causes of the SLE (Hughes, 1975; Zech et al, 1979). The renal involvement in SLE ranges from about 50% to 70% (Hughes, 1975; Leaf and Cotran, 1980).

The histological changes are divided into 4 main categories, and the clinical presentation which indicates renal involvement varies according to these changes:

1. Minimal changes or mesangial lupus nephritis. In this group the patients usually present with microscopic haematuria with slight proteinuria, and normal renal function. The urinary abnormalities may be absent in some instances (Baldwin et al, 1977).

Pathology

Light microscopy

The glomeruli may be normal or there may be a slight increase in the mesangial cells and mesangial matrix (Baldwin et al, 1977).

Electron microscopy

Mesangial dense deposits can be seen in all glomeruli (Baldwin et al, 1977).

Immunofluorescence

IgG and C3 deposits can be seen in the mesangial area. IgA and IgM can be seen sometimes (Baldwin et al, 1977; Andres et al, 1978).

Prognosis

The lesion may remain stable and the patient may live for years without any deterioration of the renal function. In some other patients the lesion progresses into other histological forms (Baldwin et al, 1977).

2. Diffuse proliferative lupus nephritis

In this group patients usually present with nephrotic or nephritic syndrome, or both. Hypertension is common (Leaf and Cotran, 1980). Proteinuria was reported in all Baldwin et al (1977) patients with this lesion and heavy microscopic haematuria occurred in the majority of these cases.

Pathology

Light microscopy

All glomeruli are usually affected. Diffuse mesangial and endothelial cell proliferation can be seen, this may be accompanied by exudative changes. "Wire loop" lesions, i.e. local regions of greatly thickened capillary wall, and fibrinoid necrosis may coexist. Epithelial crescents are common and are usually small (Burkholder, 1974; Baldwin et al, 1977). Some glomeruli may appear sclerotic. Tubular atrophy and

vasculitis may be seen (Andres et al, 1978).

Electron microscopy

Electron-dense deposits can be seen in the sub-endothelial and mesangial areas, and sometimes the deposits can be seen in sub-epithelial and intramembranous areas; they are usually large in size (Baldwin et al, 1977; Burkholder, 1974).

Immunofluorescence

Diffuse granular deposits of IgG and C3 can be seen along the glomerular capillary walls. IgM and IgA may show positive staining (Baldwin et al, 1977).

Prognosis

Remission may occur with treatment but relapses are common; renal failure develops in the majority of patients with this lesion (Leaf and Cotran, 1980; Baldwin et al, 1977).

3. Focal proliferative lupus nephritis

In this type the patients usually present with recurrent haematuria and proteinuria. Hypertension occasionally occurs (Baldwin et al, 1977).

Light microscopy

This type is characterized by segmental proliferation in some glomeruli and other glomeruli show no changes. Focal proliferation of endothelial and mesangial cells, wire loop, and fibrinoid deposits may be seen. In portions of glomeruli, focal crescent and capsular adhesions can be observed (Burkholder, 1974).

Electron microscopy

Electron-dense deposits are mainly seen in the mesangial region (Andres et al, 1978).

Immunofluorescence

Granular deposits of IgG and C3 are usually present, predominantly in the mesangial areas of all glomeruli. IgM and IgA may be present

(Baldwin et al, 1977; Andres et al, 1978).

Prognosis

The course of this type is favourable, more so than the other forms, and complete remission may occur (Baldwin and McCluskey, 1968; Baldwin et al, 1977). Transition from this form to another, e.g. diffuse proliferative GN, and membranous GN has been reported (Baldwin et al, 1977).

4. Membranous lupus nephritis

In this type the patients present with proteinuria which is usually severe and may be accompanied by nephrotic syndrome. Haematuria (usually microscopic) and hypertension may occur (Baldwin and McCluskey, 1968; Baldwin et al, 1977).

Light microscopy

The histological lesions resemble those seen in idiopathic membranous glomerulonephritis (Burkholder, 1974). The glomeruli show uniform thickening of their capillary walls. Spiky projections are usually seen (Andres et al, 1978).

Electron microscopy

Fusion of the epithelial foot processes and sub-epithelial deposits can be seen by electron microscopy. Mesangial dense deposits may be seen and can differentiate this type from the idiopathic one (Andres et al, 1978).

Immunofluorescence

Granular deposits of IgG and C3 can be demonstrated along the capillary walls (Andres et al, 1978; Baldwin et al, 1977).

Prognosis

The course of this type is slow and it may take many years before the renal function declines. Remission after steroid treatments may occur (Baldwin and McCluskey, 1968; Baldwin et al, 1977).

Serological findings in SLE

Serum complement components are commonly reduced in SLE especially C4, C3 and C1q (Ruddy et al, 1975; Leibowitch et al, 1980). An antinuclear factor is present in the great majority of cases. Antibodies to DNA also occur and generally correlate with disease activity (Hughes, 1971, 1975).

Renal lesions in subacute bacterial endocarditis

Diffuse glomerulonephritis accompanies this condition and appears to be immunologically mediated. The complement system is activated either by immune complexes or by direct action of the causative organism antigens (Boulton-Jones et al, 1974; Pertschuk et al, 1976).

Clinical presentation

Macroscopic or microscopic haematuria is the most important sign which indicates renal lesion (de Wardener, 1973). Nephrotic syndrome and progressive renal failure may occur in some patients (Boulton-Jones et al, 1974).

Pathology

It seems that the renal lesion depends on the size of the immune complexes. Boulton-Jones et al (1974) reported that small complexes formed in antigen excess were localized in a sub-epithelial position and were associated with diffuse proliferation, whereas larger complexes formed in antibody excess were localized on the inside of the glomerular basement membrane and were accompanied by a focal nephritis. According to this, the renal lesion is divided into two types:

1. Acute diffuse glomerulonephritis

This type is similar to the lesion in acute post-streptococcal glomerulonephritis. Microscopically there is proliferation of the endothelial and mesangial cells, accompanied by polymorph leukocyte infiltration (Tu et al, 1969). Sub-epithelial and sub-endothelial electron-dense deposits can be seen by electron microscopy (Pertschuk et al, 1976; Tu et al, 1969). By immunofluorescence, diffuse granular deposits of complement with or without IgG and IgM can be seen in the glomerular loop (Pertschuk et al, 1976; Boulton-Jones et al, 1974).

2. Focal proliferative glomerulonephritis

Focal and segmental proliferation of the endothelial and mesangial cells can be seen by light microscopy, focal crescents and capsular adhesions occasionally occur. Sub-endothelial deposits can be demonstrated by electron microscopy (Burkholder, 1974). Diffuse granular pattern of IgG, IgM and complement are usually demonstrated on the glomerular capillary wall and mesangial area by immunofluorescence (Boulton-Jones et al, 1974).

Serological findings

Circulating immune complexes can be detected in most cases, rheumatoid factor titres are often elevated, and serum concentration of complement components are often depressed (Bayer et al, 1976; Mohammed et al, 1977).

Prognosis

Successful eradication of the infection will often reverse renal disease unless it is far advanced (Gutman et al, 1972; Boulton-Jones et al, 1974; de Wardener, 1973).

Post-staphylococcal glomerulonephritis (Shunt nephritis)

The association of nephritis with an infected ventriculoatrial shunt in hydrocephalic patients was reported by Black et al (1965). Their patients, two children with ventriculoatrial shunt, presented with nephritic syndrome and bacteraemia due to coagulase-negative staphylococcus.

This was followed by a report by Stickler et al (1968) in which they reported 6 cases with diffuse glomerulonephritis and associated infected ventriculoatrial shunt. The infecting organism was a coagulase negative staphylococcus in all but one patient with coagulase positive staphylococcus.

Clinical presentation

Patients may present with nephrotic or nephritic syndromes and gross haematuria is common (Stickler et al, 1968).

Pathology

Light microscopy

Renal biopsy shows a diffuse GN with proliferation of the endothelial and mesangial cells with glomerular lobulation. Polymorph leukocyte infiltration can be seen, and thickening of the basement membrane is usually observed (Dobrin et al, 1975; Sato et al, 1979).

Electron microscopy

Granular dense deposits can be seen on the sub-endothelial area (Dobrin et al, 1975).

Immunofluorescence

Granular deposits of IgG, IgM and C3 are usually seen along the glomerular basement membrane and in the mesangial area (Dobrin et al, 1975). Staphylococcus antigen is detected in the kidney tissue in some instances (Sato et al, 1979).

Serological findings

The serological findings of this disorder are similar to those of infective endocarditis. Serum complement components are often low. The rheumatoid factor titres are often elevated. Circulating immune complexes can be detected (Dobrin et al, 1975; Pertschuk et al, 1976; Sato et al, 1979).

Prognosis

After removal of the infected shunt, the course of the disease is usually regressive (Stickler et al, 1968; Dobrin et al, 1975; Black et al, 1965).

Henoch-Schönlein purpura and the kidney

This disease is principally a disease of children and young adults but it can occur at any age (Fillastre et al, 1971; Ballard et al, 1970). It is presumed to be immunologically mediated and characterised by vasculitis, purpura, arthritis and involvement of other organs, e.g. the kidney and gastrointestinal tract (Ballard et al, 1970; de Wardener, 1973). In some instances it may be preceded by drug ingestion, e.g. tetracycline, acetyl salicylic acid and penicillin or by upper respiratory tract infections (Kobayashi et al, 1977; Ballard et al, 1970). The majority of cases give no history of any cause (Kobayashi et al, 1977). Renal manifestations include; macroscopic haematuria in most cases but microscopic in others; proteinuria, which may be mild or sometimes may be severe enough to cause a nephrotic syndrome; hypertension may also occur (Ballard et al, 1970). The renal changes observed by light microscopy may be divided into 4 main groups and the prognosis varies according to these changes:

1. Normal or minimal changes

The renal biopsy is usually normal or may show a slight mesangial proliferation in a few glomeruli. The prognosis of those patients is good (Kobayashi et al, 1977; Counahan et al, 1977).

2. Acute diffuse proliferative type

The histological changes are similar to those seen in acute post-streptococcal GN. There is usually slight proliferation of endothelial and mesangial cells. Complete remission may occur in patients with this lesion (Counahan et al, 1977; Ballard et al, 1970).

3. Diffuse GN with proliferation of the epithelial cells lining Bowman's capsule and crescent formation in the majority of the glomeruli. The lesion is usually progressive and end-stage renal failure occurs (Counahan et al, 1977; Kobayashi et al, 1977).

4. Focal GN

This is the most common histological change seen in HSP. It is characterized by focal proliferation of endothelial and mesangial areas (Kobayashi et al, 1977). The prognosis of this type is usually good and patients go on to remission and sometimes complete healing (Kobayashi et al, 1977; Fillastre et al, 1971).

Electron microscopy

Electron-dense deposits in granular pattern can be seen in mesangial and endothelial areas (Kobayashi et al, 1977).

Immunofluorescence

IgA, IgG and complement can be seen mainly in the mesangial area (Morel-Maroger, 1976; Fillastre et al, 1971).

Serological findings

Serum IgA levels may be elevated in some cases (Whitworth et al, 1976). Serum complement levels are nearly always within normal limits (Ballard et al, 1970; Kobayashi et al, 1977). Circulating immune complexes may or may not be found (Pussell et al, 1978; Woodroffe et al, 1977).

Goodpasture's syndrome

This syndrome is characterized by episodes of pulmonary haemorrhage and haematuria, associated with severe progressive glomerulonephritis. The lesion is mediated by antibodies directed toward the glomerular and pulmonary basement membrane (Benoit et al, 1964; Canfield et al, 1963; Leaf and Cotran, 1980).

The aetiology of this disease is unknown; it may follow influenza infection (Wilson and Smith, 1972; Perez et al, 1974), penicillamine treatment (Sternlieb et al, 1975), or may follow hydrocarbon inhalation (Beirne and Brennan, 1972). These factors may cause pulmonary damage and allow the circulating antibodies to react with the alveolar basement membrane which is antigenically similar to the glomerular capillary basement membrane and therefore the antibodies produced cross react with GBM which cause the renal injury.

Clinical manifestation

Haemoptysis is usually the first symptom to appear with cough and dyspnoea accompanied by a nephritic syndrome and macroscopic or microscopic haematuria. Oliguria may be found, and also proteinuria which may cause a nephrotic syndrome (Proskey et al, 1970; Canfield et al, 1963; Briggs et al, 1979).

Renal pathology

Light microscopy

The glomerular lesions vary from focal proliferation to diffuse proliferation with crescent formations (Briggs et al, 1979). The earliest lesion shows focal proliferation in the tuft. Fibrinoid necrosis is usually seen within the tuft (Proskey et al, 1970). Epithelial cell proliferation occurs at the affected area at first then the proliferation spreads to involve the whole tuft with crescent

formation. This progesses to fibrosis and capsular adhesion. The glomerular basement membrane may be thickened (Berlyne, 1979; Morel-Maroger, 1976). Tubules may be dilated in some patients and atrophied in others (Benoit et al, 1964; Proskey et al, 1970).

Electron microscopy

By electron microscopy, endothelial cell proliferation can be seen and may fill the capillary lumen. There is hypertrophy of the mesangial cells, and the mesangial area appears large (Burkholder, 1974). The basement membrane materials are increased and the foot processes are usually lost. Epithelial pseudo-villi may be seen, fibrin may be present beneath the endothelium (Proskey et al, 1970; Heptinstall, 1974).

Immunofluorescence

Diffuse deposition of IgG with or without C3 along the GBM in linear pattern is usually found in this disease (Brigges et al, 1979; Perez et al, 1974).

Serological findings

Serum complement components remain normal or fall with the development of end-stage renal failure (Glassock, 1979). Circulating antiglomerular basement membrane antibody can be found in the majority of patients with this syndrome. Circulating immune complexes are not found (Glassock, 1979; Woodroffe et al, 1977).

Prognosis and treatment

The disease is usually progressive and fatal (Benoit et al, 1964), spontaneous remission may occur (Wilson and Smith, 1972), but it is uncommon. Patients may die from pulmonary insufficiency or renal failure (Benoit et al, 1964), although haemodialysis may maintain the patient's life. Immunosuppression and steroids may or may not induce remission. Seaton et al (1971) reported remission in two patients

treated by steroids and immunosuppression. Lockwood et al (1975) reported recovery in patients with Goodpasture's syndrome after treatment with plasmapheresis and immunosuppressive drugs.

Polyarteritis nodosa and the kidney

This is an inflammatory condition which affects medium and small arteries throughout the body. The lesion may be seen in any organ or tissue. Renal involvement occurs in the majority of cases and death from renal failure is common (de Wardener, 1973; Baldwin and McCluskey, 1968). Australia antigen can be found in about 50% of these patients (Trepo et al, 1974) which denotes that immune complexes may be responsible for the mechanism of this disease. The early report came when Gocke et al (1970) reported four patients positive for Australia antigen out of eleven with PAN and immunofluorescent studies of tissue from one patient showed deposition of Australia antigen, IgM and C3 in blood vessel walls.

Clinical presentation

The patients present with microscopic haematuria and an acute nephritic or nephrotic syndrome. Hypertension is usual. Other patients may present with ARF (de Wardener, 1973); Darmady et al (1955) reported renal tubular damage in patients with PAN.

Pathology

Light microscopy

In the macroscopic form, the involvement of the kidney is manifested by necrotizing changes in the arteries of different sizes, either with or without necrotizing and proliferative glomerular lesions (Andres et al, 1978).

In the microscopic form, the most conspicuous changes are in glomeruli which show areas of fibrinoid necrosis restricted to a few lobules or affecting the entire tuft. This may be accompanied by crescent formation and capsular adhesions with polymorph infiltration (Andres et al, 1978; Heptinstall, 1974).

Electron microscopy

In some patients electron-dense deposits have been seen both beneath the BM and in subepithelial areas (Heptinstall, 1974; Andres et al, 1978).

Immunofluorescence

In a few patients IgG, IgM and C3 may be found in a granular pattern along the glomerular capillary walls. Australia antigen (HbsAg) may be indentified in the walls of the vessels of some patients (Andres et al, 1978). Fibrinogen may be the only constituent in other patients (Heptinstall, 1974; Andres et al, 1978).

Serological findings

Australia-antigen or antibody may be found in the serum of patients, and circulating immune complexes detected in some cases (Gocke et al, 1970; Trepo et al, 1974; Woodroffe et al, 1977).

Prognosis

The renal lesion may remit and relapse although progession to renal failure with death from end-stage renal failure may occur (Spencer and Rose, 1957). The prognosis has become better since the use of high dose corticosteroids (de Wardener, 1973; Berlyne, 1979).

Malaria and the kidney

The association between quartan malaria and nephrotic syndrome has been known for many years. Gilles and Hendrickse (1963) and Kibukamusoke et al (1967) reported that the majority of African children who presented with nephrotic syndrome, had Plasmodium malariae in their peripheral blood. It has been suggested by Gilles and Hendrickse (1963) that the renal damage is due to the formation of immune complexes. This is confirmed by the observation of Ward and Kibukamusoke (1969) and Allison et al (1969) in which they demonstrated the presence of complexes in the kidney of children with malarial nephropathy. McGregor et al (1968) isolated soluble antigens from the serum of patients with Plasmodium falciparum malaria.

Clinical presentation

Patients usually present with a nephrotic syndrome. Severe oedema is usually present (Gilles and Hendrickse, 1963; Kibukamusoke et al, 1967). Proteinuria is usually heavy and of non-selective type; haematuria is microscopic although macroscopic haematuria may occur less often, and granular casts may be present (Gilles and Hendrickse, 1963; Kibukamusoke et al, 1967).

Pathology

Light microscopy

The renal lesion is characterized by capillary-wall thickening and segmental glomerular sclerosis, or by focal proliferative GN with variable focal and segmental proliferation of the endothelial and mesangial cells; crescent formation is unusual (Edington, 1967; Gilles and Hendrickse, 1963; Tighe, 1975). Voller et al (1971) reported a generalized diffuse glomerulonephritis with proliferative mesangial and membranous changes in a kidney at autopsy from a monkey infected

with Plasmodium malariae.

Electron microscopy

Granular electron-dense deposits can be seen on the endothelial surface. Sub-epithelial deposits are not common in quartan malarial nephropathy but Allison et al (1969) reported sub-epithelial deposits in some biopsy specimens. Endothelial and mesangial cell proliferation can be seen. The basement membrane is moderately thickened and there is fusion of the epithelial foot processes (Tighe, 1975).

Immunofluorescence

IgM, IgG and C3 are usually present along the capillary basement membrane in granular pattern. IgM is usually more common than other immunoglobulins (Tighe, 1975; Ward and Kibukamusoke., 1969). Malarial antigens are detectable in the glomeruli in some cases (Ward and Kibukamusoke, 1969).

Prognosis

Patients with malarial nephropathy show little or no response to antimalarial therapy or immunosuppressive agents (Glassock, 1979; Kibukamusoke et al, 1967).

Secondary syphilis and the kidney

Some patients develop renal lesions during the course of secondary syphilis, presenting with a nephrotic syndrome (Robins and Ladd, 1962). It has been reported that the renal injury is mediated by antigen-antibody reactions (Falls et al, 1965). Warthin (1922) reported the presence of the spirochaetes within the tubules of the kidneys.

Pathology

Light microscopy

Proliferation of the endothelial and mesangial cells which leads to glomerular hypercellularity and endothelial enlargement can occasionally be seen (Braunstein et al, 1970). Focal thickening of the capillary wall may be seen. Some loops of the PCT may contain hyaline droplets (Falls et al, 1965).

Electron microscopy

The endothelial and mesangial cells show slight swelling and there may be focal swelling of the BM. The most conspicuous feature is the presence of sub-epithelial "humps" like electron-dense deposits (Braunstein et al, 1970). Extensive fusion of the epithelial cell foot processes can be demonstrated (Falls et al, 1965).

Immunofluorescence

IgG was demonstrated by Braunstein et al (1970) in the capillary loops of the glomeruli, but they failed to demonstrate IgM, IgA or complement.

Prognosis

All of these changes regress and the kidney may return to normal during antisyphilitic therapy (Falls et al, 1965).

Immunity to Infection

Immunity is the state of response to, recognition of and elimination of antigens, and it therefore gives resistance to the body against the invasion of pathogens. Immunity is divided into natural (innate or non-specific) immunity, and acquired (specific) immunity (Roitt, 1980; Herbert and Wilkinson, 1977).

A. Natural (innate or non-specific) immunity

This forms the first line of protection against infections and is associated with the following factors:

Age: The susceptibility of the very young and very old groups to infection; for example, pneumonia is more likely to be fatal in the elderly, as is septicaemia with E.coli in the newborn (Bellanti, 1978).

Race: e.g. Negroes are more susceptible to tuberculosis than whites (Bellanti, 1978).

Hormonal factors: e.g. Susceptibility to infection in patients treated with corticosteroids and patients with diabetes mellitus (Bellanti, 1978).

Anatomic factors: The first line of defence against infection is usually provided by the skin and the mucous membranes (Bellanti, 1978).

Biochemical factors:

a. Opsonins - factors present in plasma and other body fluids which bind to microorganisms and make them more susceptible to ingestion by phagocytes (Drutz and Mills, 1980). They may be: (1) Antibody, (2) Activated products of complement component, (3) Non-antibody, non-complement factors (Herbert and Wilkinson, 1977).

b. Lysozyme - the enzyme present in tears, saliva and nasal secretion has bacteriocidal activity and potentiates the action

of the complement on bacteria. It has been called a natural antibody (Bellanti, 1978; Drutz and Mills, 1980).

c. The complement system - this is an important effector system which consists of a number of separate plasma proteins and plays an important role in the host's defence against infections.(Ruddy et al, 1972; Lachmann, 1975). The complement system may be activated through two routes:

- The classical pathway is initiated by antigen-antibody interaction. Activation starts at the first complement component, C1, when it binds to the Fc portion of antigen-antibody complexes (usually IgM or IgG)(Lachmann, 1975).

- The alternative pathway (properdin pathway) is activated by many substances such as gram negative endotoxin, a factor from cobra venom, zymosan and heat-aggregated IgA. Activation starts at C3 (Lachmann, 1975; Ruddy et al, 1972).

The complement activation results in the following biochemical events:

i Chemotaxis. This seems to be the function of the activated complements C567 as well as C3a and C5a. Polymorphonuclear leukocytes and possibly macrophages show chemotaxis towards these components (Drutz and Mills, 1980; Brown, 1975).

ii Immune adherence. This is a function of C3b which binds to macrophages, neutrophils, and T and B lymphocytes by the C3b receptors on the walls of these cells, thus enhancing phagocytosis (Brown, 1975; Ruddy et al, 1972).

iii Anaphylatoxin. C3a and C5a cause the degranulation of mast cells and basophils, causing the liberation of vasoactive amines, e.g. histamine. These substances increase the capillary permeability, act as vasodilators and lead to the accummulation of neutrophils (Ryan and Majno, 1977; Brown, 1975).

iv Cell lysis. This is due to the action of the activated C5-C9 (Ruddy et al, 1972; Brown, 1975).

d. Interferon - this is a non-immunoglobulin protein which is produced in response to viral infection by almost all nucleated cells. It stimulates cells to produce a second protein which blocks translation of viral messenger RNA, and thus protects them from infection. It is a non-specific anti-viral agent as it can act on all viruses, but viruses can escape from the effect of interferon (Herbert and Wilkinson, 1977; Fenner and White, 1976).

e. Physiological factors - gastric juice can destroy some ingested bacteria (Bellanti, 1978).

f. Cellular factors - e.g. macrophages and polymorphonuclear leukocytes. These cells are characterized by their capacity to phagocytose foreign particles by a process called phagocytosis (cell eating) (Bellanti, 1978).

The steps of phagocytosis are the following:

i - Chemotaxis. Movement toward the object.
ii - Attachment to the object usually following its opsonization.
iii - Ingestion by vacuole formation, killing and destruction of the microorganism (Bellanti, 1978; Drutz and Mills, 1980).

There are a number of killing mechanisms operating in the phagolysosomes, four of which are:

- Low pH and lactic acid.
- Phagocytins (a group of basic proteins lethal for many gram-positive and gram-negative organisms.
- Lysozyme (a major lysosomal enzyme which can lyse some gram-positive organisms.
- Hydrogen peroxide (which is produced by the oxidation of glucose through the hexose monophosphate shunt in conjunction with halide

ions (Cl^- or I^-), and myeloperoxidase. This is an important mechanism for phagocytic killing of many bacteria and fungi (Bellanti, 1978; Drutz and Mills, 1980).

B. Acquired (specific) immunity

1. Passive immunity: By transferring immunity from an immune to a non-immune person. This may be:
   a. Natural: This immunity is transferred from the mother to the neonate through the placenta (IgG) and colostrum (IgA)(maternal immunity).
   b. Artificial: By giving gamma globulin or antitoxin, as in the treatment of immunodeficient states, tetanus and diphtheria (Herscowitz, 1978; Janeway and Rosen, 1966).
2. Active immunity: The immune response of the body which gives protection of the individual against infection. This may be
   a. Natural, e.g. The immune response following infection
   b. Artificial, e.g. After deliberate vaccination (Herscowitz, 1978)

The components of the specific immune response

There are two types of effector mechanisms that mediate specific immune responses:

1. Cell mediated immunity: This is mediated mainly by T-lymphocytes.
2. Humoral immunity: This is mediated by B-lymphocytes.

T-lymphocytes (cell mediated immunity)

T cells arise in the bone marrow and differentiate in the thymus. They possess specific receptors for antigen (Marchalonis, 1975). On contact with the appropriate antigen these lymphocytes mature into:

a. Helper T cells. These cells co-operate with B cells in antibody formation (Paul and Benacerraf, 1977).

b. Suppressor T cells: These cells are able to suppress the action of specific B cells and so prevent their differentiation into antibody producing cells (Paul and Benacerraf, 1977).

c. Cytotoxic T cells: These cells have cytotoxic activity with target cells (Bellanti, 1978).

d. Memory cells: These are generated during the initial exposure to the antigen; secondary responses are dependent on the existence of these cells (Herscowitz, 1978).

Activated T cells can kill target cells either directly or through the elaboration of soluble factors known as lymphokines. Some of the most important lymphokines are macrophage inhibition and activation factors, chemotactic factors, transfer factor, lymphotoxin, cell growth inhibitors and interferon (Irvine, 1979; Herbert and Wilkinson, 1977).

Killer cells

Another cellular type important in cell mediated reactions is the killer or K cell, which is capable of killing target cells coated with IgG antibody in an antibody-dependent cellular cytoxicity (ADCC) mechanism. The nature and origin of these cells are still debated (MacLennan and Harding, 1974; Bellanti, 1978).

B-lymphocytes

B cells are derived from the bone marrow (or bursa of fabricius in birds). These cells possess specific receptors for antigens (Marchalonis, 1975). When B cells are stimulated by an antigen they differentiate into memory cells and plasma cells. The latter are the major antibody synthesizing cells (the structure and function of antibodies are discussed below). Thus B cells are responsible for humoral immunity (McConnell, 1976; Herscowitz, 1978).

B-lymphocytes and thymus dependent antigens

Both B and T cells have specific receptors for antigens on their surfaces. Certain antigens are capable of reacting directly with the corresponding antibody (usually of IgM class) on the surface of a B cell to induce it to transform into active plasma cells. In the case of thymus-dependent antigens such as haptens and simple proteins in some cases, antibody production (usually of IgG class) seems to require T cell help (McConnell, 1976; Paul and Benacerraf, 1977).

Structure and function of antibodies

Antibodies are high molecular weight proteins secreted mainly by plasma cells in response to stimulation by antigen and have the molecular properties of immunoglobulins. They are present in the serum in different amounts which may vary considerably in disease (Nezlin, 1977; Herbert and Wilkinson, 1977). In man there are five main classes of immunoglobulins, IgG, IgM, IgA, IgD and IgE.

Structure of antibodies

The immunoglobulin molecule consists of four polypeptide chains, two identical heavy and two identical light chains, which are formed of amino acids linked together by peptide bonds (Porter, 1967, 1973). Each chain consists of two regions known as the variable amino-terminal portion and constant carboxyl-terminal portion, each of which in turn is determined by at least one gene (Nezlin, 1977; Goodman and Wang, 1980). The four chains are held together by disulphide bonds (Porter, 1967).

The light chains are of two types, Lambda (L) and Kappa (K), of which individual immunoglobulins have only one type (Nezlin, 1977; Porter, 1973).

The heavy chains are of five main types each of which gives rise to a distinct immunoglobulin class and identified by the Greek-letter equivalent of their class name, γ in IgG, μ in IgM, α in IgA, δ in IgD and Σ in IgE (Goodman and Wang, 1980; Nezlin, 1977).

The enzyme papain splits the $Ig\begin{matrix}G\\ \lambda\end{matrix}$ molecule into three fragments, two identical fragments known as Fab (for fragment antigen binding) and the third known as Fc (crystallizable fragment) (Porter, 1967, 1973). The heavy chains each have a central flexible hinge area on which the Fab fragments can rotate (Goodman and Wang, 1980). The Fc fragment

of the heavy chain is responsible for the antigenic difference between the classes of immunoglobulins (Low et al, 1976; Irvine, 1979). Digestion of immunoglobulin with a proteolytic enzyme, pepsin, leaves two Fab molecules held together by a disulphide bond and called $F(ab)_2$ (Porter, 1967; Nezlin, 1977).

Function of antibodies

Immunoglobulin G(IgG)

IgG comprises about 75% of the total serum immunoglobulins. It has a molecular weight of 140,000-150,000 and a sedimentation coefficient of 7S; it is distributed equally between the blood and interstitial tissues (Nezlin, 1977; Turner, 1977). IgG antibodies are most often formed as the secondary response to an antigenic stimulus (van Oss, 1979). They can pass through the placental barrier from mother to foetus and provide the neonates with passive immunization during early life (Irvine, 1979). IgG antibody is a highly effective opsonin and can act as an antitoxin (neutralising antibody)(van Oss, 1979). There are 4 subclasses of IgG: IgG1, IgG2, IgG3 and IgG4 and their biological function differs. IgG1 and IgG3 fix complement via the classical pathway and bind to macrophages. IgG2 fixes complement only weakly, IgG4 is unable to fix complement by the classical pathway (Goodman and Wang, 1980). The four subclasses can react with rheumatoid factor and cross the placenta (Turner, 1977; Irvine, 1979; Goodman and Wang, 1980).

Immunoglobulin M(IgM)

IgM is found mainly in the serum with a molecular weight of 900,000 and a sedimentation coefficient of 19S. It has a star shape with five identical pentameric structures joined together by J chain (Nezlin, 1977), it is the earliest antibody response to a primary immune stimulus (Turner, 1977). IgM titres rise rapidly within days of infection but decline after

a few weeks. IgM does not pass the placenta, ..fixes complement but not rheumatoid factor, acts as a powerful agglutinating antibody (van Oss, 1979), and plays an important role in the elimination of large microorganisms from the circulation (Turner, 1977). IgM antibodies can be made without T cell help (Irvine, 1979).

Immunoglobulin A(IgA)

The IgA molecule is found both in serum and in external secretions (e.g. saliva, colostrum, tears and in the mucus of respiratory, intestinal, and genito-urinary tracts)(Nezlin, 1977; Turner, 1977). In serum it is monomeric with a molecular weight of 155,000 and sedimentation coefficient of 7S (van Oss, 1979), while in secretions it is usually in dimeric form with a molecular weight of 400,000 and sedimentation coefficient of 11S (Goodman and Wang, 1980). The polymeric units of secretory IgA are joined together by a J chain and a secretory piece (Nezlin, 1977; Turner, 1977). The IgA molecule does not cross the placenta and does not fix rheumatoid factor nor complement by the classical pathway, although the aggregated form can activate the alternative pathway (Goodman and Wang, 1980; Turner, 1977). Direct stimulation of mucous surfaces with antigen leads to the formation of secretory IgA which can neutralize viruses and can inhibit attachment of bacteria to epithelial cells (Williams and Gibbons, 1972; Turner, 1977).

Immunoglobulin D(IgD)

The IgD molecule is a monomer, it has a molecular weight of 184,000 and a sedimentation coefficient of 7-8S. It is present in serum in small amounts. The main function of IgD has not yet been determined (Goodman and Wang, 1980; van Oss, 1979), but is a surface component of lymphocyte membranes.

Immunoglobulin E(IgE)

The IgE molecule has a molecular weight of 187,000 and a sedimentation coefficient of 8S (van Oss, 1979). It includes most of the reaginic antibody and is present in serum in a very low concentration. The combination of antigen (usually called allergen) with IgE molecules on the surface of the mast cell triggers the release of vasoactive amines which initiate immediate hypersentivity (Turner, 1977). IgE levels may be increased ten times or more in patients with allergic diseases (van Oss, 1979). IgE may function in the defense against parasitic infection especially intestinal helminths (van Oss, 1979).

STRUCTURE AND FORMATION OF IMMUNE COMPLEXES

A. The Constituents of Immune Complexes

Immune complexes (IC) are macromolecular complexes formed as the result of the combination of antigen and antibody. The complexes vary greatly in size and structure depending on the size of the antigen, the class and avidity of antibody, the incorporation of complement components, and the formation of a lattice work structure. The formation of immune complexes is a normal part of the immune response, but in healthy individuals these are cleared by the reticuloendothelial system. Failure to clear may cause immune complexes to be deposited in tissues and lead to immune complex disease (WHO Scientific Group, 1976; Carter, 1973; Maini, 1979).

The antigens involved in IC formation are proteins, nucleoproteins, polysaccharides, DNA and RNA, derived from viruses, bacteria, fungi and parasites, as well as from body constituents (WHO Scientific Group, 1976; Theofilopoulos and Dixon, 1979; Hughes, 1975; Roitt, 1980). Parenteral or oral administration of drugs can be a non-infectious source of antigens for IC formation. The binding of IC to the C1q part of the first complement component can trigger activation of the complement sequence, and result in the incorporation of different complement fragments in the complexes. Binding of the IC to cell membranes can be either by Fc receptors or C3b receptors. Human macrophages and neutrophils have receptors for C3b, C3a and C5a, and eosinophils for C3a, C3b, C3d and C5a (WHO Scientific Group, 1976). Fc receptors are present on all these cells and also on human platelets.

B. Composition of Immune Complexes

(i) Lattice Structure When an antigen solution is mixed in the correct proportions with antiserum a precipitate is usually formed. As more and

more antigen is added an optimum is reached after which further addition of antigen leads to progressively decreasing amounts of precipitate. At this stage the supernatant can be shown to contain soluble complexes of antigen (Ag) and antibody (Ab) mostly of composition Ag_4Ab_3, Ag_3Ab_2 and Ag_2Ab. Between these extreemes the cross-linking of antigen and antibody will generally give rise to three dimensional lattice structures, which coalesce to form large precipitating aggregates (Roitt, 1980). The antigen-antibody interaction is reversible, especially with monovalent antigens, and involves hydrophobic hydrogen bonds, van der Waal's forces and electrostatic bonds. As at least part of antigen-antibody interactions are due to electrostatic bonds, extremes of pH which make the proteins either all positively or all negatively charged make them lose their capacity for attraction and may even result in charge repulsion. The addition of a large number of counterions combining with the charged groups may also lead to an increased rate of dissociation of the antigen-antibody bonds. Thus high salt concentrations may also dissociate antigen-antibody complexes which are partly or largely electrostatically bonded. Dissociation is most frequently carried out at low pH (3 - 3.5) (van Oss, 1979).

The solubility of complexes is dependent on the macromolecular structure of the polymerised antigen-antibody union. In the simplest forms, where all the binding sites of the antigen are covered with antibody molecules, or alternatively when both (IgG) antibody binding sites are occupied with antigen, the complexes are small and soluble. These are of composition $Ag\text{-}Ab_n$ and $Ag_2\text{-}Ab$ respectively.

The simplest polymers produced would be linear ones of the form Ag-Ab-Ag-Ab-Ag. With antigen and antibody valencies of 2, only these linear polymers can be formed. Huge linear polymers of this kind can stay in solution. An example would be DNA which can have molecular weights in the range of

100 million daltons and still be soluble. If the antigen, whose valency can differ from complex to complex, has a valency of two, the complexes formed must all be linear. In vitro experiments have established that with antigens of low valency (less than three) precipitation of complexes is not found.

When the valency of the antigen is greater than two cross-linking of the polymers is possible, and it is not surprising that with equivalence ratios of antigen and antibody that are large cross-linked three dimensional lattices are formed and fall out of solution. Obviously this might also happen with IgM antibodies and antigens which only had a valency of two. The dimer of IgA with a valency of four might also be able to make precipitating complexes with bivalent antigens.

(ii) Involvement of Rheumatoid Factors Rheumatoid factor is an antibody, usually of the IgM class; IgG and IgA rheumatoid factors do occur in lesser amounts. Rheumatoid factor binds rather loosely with native IgG, but much more tightly with aggregated IgG. Whether the aggregation is produced by antigen or other factors such as heat, the binding of the rheumatoid factor is increased when several bonds are made between the rheumatoid factor and different aggregated Fc regions of IgG, compared with the single bond which can occur to native IgG.

Rheumatoid factor occurs often in patients with rheumatoid arthritis, but may occur in many other conditions, probably as a result of immunisation with circulating IC. IgM rheumatoid factor produces complexes with native IgG of a sedimentation constant of about 22 (van Oss, 1979), but may react with IC and become a component of the IC increasing its size and complexity. The rheumatoid factor may,

by binding to the Fc regions of the complexed IgG, to some extent interfere with the binding of Clq and hence affect complement activation by the complex.

(iii) Involvement of Complement Components Complement components which interact with and bind to IC are the early components of the classical pathway, Cl, C4, C2 and C3. In addition if alternative pathway activation occurs factor B and C3b of the amplification loop may also be present in significant amounts. The quantities of the components depend on the degree of activation of the pathways occurring, but may represent more than 50% of the protein in some immune complexes. The Cl macromolecule consists of one Clq molecule of 400,000 daltons and a tetramer to hexamer of Clr and Cls (i.e. 4 x 83,000 daltons). In addition C4, C2 and C3 have a combined molecular weight of about 500,000 daltons. Therefore the total molecular weight of these components when attached to a complex of say 75,000 antigen and 300,000 antibody could exceed one million daltons (Jones and Orlans, 1981).

(iv) Solubilisation of Immune Complexes by Complement The addition of fresh normal serum to an immune precipitate may result in its solubilisation (Takahashi et al, 1977; Czop and Nussenzweig, 1976). It has been reported that complement activation by the IC leads to the interposition of C3b molecules into the antigen-antibody lattice leading to its rearrangement into smaller and therefore more soluble complexes. This process was recognized in the course of experiments on the fate of immune complexes attached through a C3b receptor to the surface of B lymphocytes. It was found that in the presence of normal serum such complexes were released, and were no longer capable of binding to the surfaces of fresh B lymphocytes, or other C3b receptor bearing cells (Takahashi et al, 1977; Czop and

Nussenzweig, 1976).

It is now obvious that the release mechanism is the same process as the solubilisation of antigen-antibody precipitates. It has been shown that in rabbits with acute serum sickness, depletion of complement by cobra venom factor delayed the removal of glomerular-bound radiolabelled antigen (Bartolotti and Peters, 1978, 1979). Takahashi et al (1977) have reported that during the solubilisation of BSA/anti-BSA immune precipitates by human complement at least three stages can be distinguished. First is the degeneration of alternative pathway C3 convertase sites associated with the complexes. During the first few minutes of interaction between the immune aggregates and serum, before any solubilisation has taken place, properdin factor B and C3 moieties are incorporated into the lattice. The washed precipitates have C3 convertase activity, which can be inhibited by antibodies to factor B, but not by antibody to C2.

The assembly of the convertase is temperature dependent and does not take place in the absence of Mg ions. Thus it appears that it is due to alternative pathway activation. The immune complex associated C3 convertase activity decays rapidly at 37C, but can be restored by addition of purified factor B and properdin. An amplification step occurs next; C3 convertase bearing aggregates are incubated with purified C3, and solubilisation takes place. It appears that solubilisation is caused by the accumulation of a large number of C3 fragments on the Ag-Ab lattice. In solubilized complexes, the molar ratios of Ab/C3 are close to one. The final step in the solubilisation process is a secondary reaction, during which some rearrangement of the lattice takes place. It occurs in medium devoid of serum, and does not require divalent cations.

(v) Effect of Composition on Clearance In experimental animals phagocytosis

by macrophages, polymorphs, eosinophils etc., is the normal fate of large complexes, whereas complexes which are smaller or non-complement fixing are cleared to some extent by the spleen (WHO Scientific Group, 1976; Mannick and Arend, 1971). The attachment of immune complexes to macrophages is promoted by complement, but ingestion requires an intact Fc region of the IgG in the complexes. If the class of antibody in IC is such that it does not fix complement or does not interact with cellular Fc receptors then the immune complexes may not be eliminated by the phagocytic system and thus may be deposited in tissues, with resulting injury (Theofilopoulos and Dixon, 1979). The activation of complement by IC leads to the production of C3b which binds to receptors on macrophages and neutrophils, thus enhancing phagocytosis of the IC. Beta-1-h and KAF can cleave C3b so that proteolytic enzymes can cut off the C3d fragment. If this happens to complexes, the C3d fragment which remains bound cannot interact with C3b receptors, and clearance by phagocytes may be small (Thompson, 1978).

Immunity to bacteria

When bacteria enter the body the immune response involves the interaction of T cells, B cells and macrophages (Bellanti, 1978).

Role of humoral antibody:

Humoral antibodies play a major role in the defence of the body against bacterial infection. They protect against infection in several ways. Antibodies can neutralize toxins of certain bacteria, for example, diphtheria and tetanus, and stop them from interacting with target organs (Allison, 1979). Antibodies and complement produce holes in the cell walls of certain bacteria; these allow the serum lysozyme to destroy the bacteria (Roitt, 1980). When a specific antibody reacts with the surface of a microorganism, the antibody-antigen complex sequentially activates the classical pathway and leads to the generation of immune adherence, anaphylatoxin and chemotactic factors. The latter are able to attract macrophages and polymorphonuclear leukocytes to the sites of bacterial invasion leading to phagocytosis and subsequent intracellular killing (Faulk and Greenwood, 1977). Opsonization of invading organisms is another function of the antibody, for it promotes phagocytosis of these organisms as discussed above (Drutz and Mills, 1980). The secretory IgA antibody appears to inhibit bacterial adherence to mucosal surface (Williams and Gibbons, 1972). It seems that IgM antibodies have greater opsonizing, bacteriocidal and agglutinating capacities than IgG, while IgG antibodies have greater precipitating capacities (Bellanti, 1978).

Cellular immunity

Some strains of bacteria such as Myobacterium tuberculosis, Myobacterium leprae, Brucella and Salmonella are able to live and multiply within macrophages. In such infections, cell mediated immunity is more important than humoral immunity as the antibody cannot reach

intracellular organisms (Turk, 1978; Faulk and Greenwood, 1977).

Sensitized T cells can destroy bacteria either directly or through the elaboration of lymphokines which attract macrophages to a site of infection, where the activated macrophages can cause intracellular killing of the organisms (Turk, 1978).

Immunity to viruses

Viruses are the smallest infectious agents and are not normally visible with a light microscope, they are only capable of replication within the living cells of a host. Viruses differ essentially in that they contain either RNA or DNA but not both (Fenner and White, 1976; Jawetz et al, 1978).

Some of the most important groups in man are:

a) DNA Viruses:

1. Herpes viruses: e.g. Herpes simplex, chicken pox, zoster and infectious mononucleosis (EB virus).
2. Pox viruses: e.g. smallpox, cow pox.
3. Adenoviruses: e.g. respiratory diseases.
4. Papoviruses: e.g. warts.

b) RNA Viruses:

1. Arboviruses: e.g. Dengue fever, Yellow fever and encephalitis.
2. Orthomyxoviruses: e.g. influenza.
3. Paramyxoviruses: e.g. parainfluenza, measles and mumps.
4. Rhabdoviruses: e.g. rabies.
5. Picorna viruses: e.g. poliomyelitis, Coxsackie and echo viruses.

Other important human viruses remain to be considered. These include viruses that cause hepatitis (hepatitis A, which may be a picorna virus, and hepatitis B, which may be a parvovirus.) and viruses responsible for certain immune complex diseases and for neurologic disorders with a long

latent period ("slow" virus disease)(Fenner and White, 1976; Jawetz et al, 1978). The immune response to viral infections involves the interplay of T and B lymphocytes (Fenner and White, 1976).

1. Humoral factors: Antibodies probably constitute one of the more important mechanisms of host resistance to viral infection (Allison, 1979; Fenner and White, 1976; Drutz and Mills, 1980). The antibody response observed in viral infections follows the same steps as observed with any other antigenic stimulus, IgM titres rise rapidly within days of infection but decline after a few weeks (Fenner and White, 1976), whereas IgG continues to be synthesized for many years and often confers protection against re-infection for life (Bellanti, 1978; Fenner and White, 1976). The serum antibody molecule can neutralize viruses by several mechanisms. It may prevent the access of viruses to the cells thereby inhibiting the subsequent intracellular multiplication; it may activate the classical complement pathway and cause lysis of the virus particles, or may promote phagocytosis with subsequent digestion of the virus in phagolysosomes (i.e. it is an opsonizing antibody)(Drutz and Mills, 1980; Roitt, 1980).

Secretory IgA antibodies in secretions from mucous membranes are important in preventing focal infection. The secretory immunoglobulins have functional and structural properties different from those of the serum immunoglobulins (Bellanti, 1978).

It is probable that the humoral antibody response is more important in protection from re-infection (Turk, 1978).

2. Cell mediated defence mechanisms: Cell mediated immunity appears to be important in infections with viruses, as antibodies cannot enter the host's cells where the replication of viruses is taking place. Also viruses can evade the harmful effects of the antibody by spreading from cell to cell without passing through the extracellular fluid (Turk, 1978).

Sensitized T cells can act against viral infection by a variety of means, firstly by liberation of lymphokines which attract macrophages and polymorphonuclear leukocytes which may destroy viruses or virus-infected cells (Fenner and White, 1976); secondly sensitized T cells can cause lysis of virus infected cells; thirdly, sensitized T cells can liberate interferon which inhibits the replication of viruses within the cells and protects non-infected cells from viral infection (Drutz and Mills, 1980; Fenner and White, 1976).

Another protective mechanism against viral infection presumably occurs through K cells which are cytotoxic to virus-infected cells when coated with specific IgG antibody in an ADCC reaction (Bellanti, 1978; MacLennan and Harding, 1974).

Some viruses can persist and replicate for months or years in macrophages, lymphocytes and in nerve cells, causing persistent viral infections, e.g. herpes simplex and herpes zoster (Fenner and White, 1976).

Immunity to helminths

The protective mechanisms in helminthic infections such as flukes, tape worms and intestinal round worms seem to act at an early stage in the life cycle of the worms in man (Turk, 1978). Helminthic infections often stimulate the formation of large amounts of IgE, the combination of worm antigen with IgE antibody on the surface of mast cells triggers the release of vasoactive amines which may cause worm expulsion from the gut (Roitt, 1980; Turk, 1978). This mechanism leads to the release of eosinophil chemotactic factor which attracts the eosinophils to the site of mast cell degranulation, and these may kill the worm with the help of IgG antibody (Bellanti, 1978; Goodman et al, 1979). Cell mediated immune mechanisms are also required in helminthic infections (Turk, 1978). In schistosomiasis, where schistosome eggs stimulate marked granulomatous reactions in host tissue, e.g. liver or bladder, it seems that the major

aetiologic mechanisms responsible for this granuloma are cell-mediated (Colley, 1977). Circulating immune complexes have been detected in the sera of many patients infected with schistosomes, and these complexes may induce glomerulonephritis (Colley, 1977). The adult schistosomes evade the immune attack by antigen disguise, a mechanism by which the worms are covered with the host blood group antigens thereby obtaining protection from the immune response. This is also true for other types of adult helminths (Faulk and Greenwood, 1977; Goodman et al, 1979).

Immunity to protozoa

Immunity to malaria is mediated by humoral antibodies of the IgG class. These antibodies have been shown to be effective against the parasite in the erythrocytic phase (Turk, 1978). The exact role of cell mediated immunity in malaria is still under active investigation (Cypess, 1978). Patients with African Trypanosomiasis may exhibit a rapid marked increase in non-specific IgM globulins; this may be due to polyclonal activation of B cells by a parasite product (Cypess, 1978; Heyneman and Welch, 1980). These parasites escape from the harmful effects of the serum antibody by the following mechanisms:

a. Antigenic variation: This is a mechanism by which the parasites can change their antigen structure in each stage of the infection. Thus an immune response to one stage may be ineffective against the others (Goodman et al, 1979; Faulk and Greenwood, 1977).

b. The presence of circulating antigens of the parasites alone or in immune complex form, which may block the immune response (Goodman et al, 1979).

c. Intracellular localization of the protozoa (Goodman et al, 1979).

Immunity to toxoplasmosis involves both humoral and cellular components (Cypess, 1978). Cell mediated immunity plays an important role in the protection against visceral and cutaneous

leishmaniasis. It also prevents the spread of the parasites (Allison, 1979; Cypess, 1978). Both toxoplasma and leishmania can survive inside macrophages. The nature of any protective immunity to intestinal protozoa, e.g. Entamoeba histolytica is still unknown (Cypess, 1978).

Immunity to fungi

The cell mediated immune response is important in the protection against cutaneous fungal infections, e.g. dermatophyte infections which involve the epidermis and its appendages and can produce infections of the scalp, of the skin and of the nails. Non-specific immunity may protect against these infections, e.g. sebaceous secretions help to control ringworm infections of the scalp (Ballenti, 1978). In subcutaneous fungal infections, e.g. sporotrichosis (chronic granulomatous infection of skin, lymphatics and other tissues)(Jawetz et al, 1978), immunity mechanisms appear to include B and T cells and non-specific factors (Bellanti, 1978). Cell mediated immunity appears to be important in opportunistic fungal infections, e.g. candida albicans, which can produce systemic disease in debilitated or immunosuppressed patients (Jawetz et al, 1978; Turk, 1978).

In systemic fungal infections, e.g. cryptococcosis (cryptococcus), coccidioidomycosis (coccidiodes) and histoplasmosis (histoplasma), both humoral and cellular immunity may be involved. When these organisms enter the body through the respiratory tract or the skin both macrophages and neutrophils are able to phagocytize and destroy them, but they can live within macrophages for long periods (Bellanti, 1978).

CHAPTER TWO
MATERIALS AND METHODS

Isolation and Estimation of Immune Complexes (IC) from Human Sera

Materials

1. Standard serum taken from a pool of more than 20 sera from the ante-natal clinic, i.e. normal human serum with respect to IgM, IgG and C1q levels. Individual normal human sera were also taken from laboratory workers. All sera were stored at -70°C for not more than 3 months.

2. Test sera: Either used fresh or stored at -70°C.

3. Antisera (anti C1q, IgG, IgM and IgA) raised in rabbits by immunisation with purified components, and standardised by comparison with known reference antibody.

4. Barbitone buffered saline (BBS)

Stock solution was made up from 85g Sodium Chloride
3.75g Sodium Barbitone (BDH Chemical Ltd.)
5.75g Barbitone (Diethyl Barbituric Acid) (BDH Chemical Ltd.)
in 2L distilled water.

5. Working (BBS)

This was made by dilution of stock 1:5 with distilled water (pH at 25°C; 7.5).

6. Ethylene Diamine Tetra acetic acid (EDTA)

0.2 M EDTA made up from

74.44 g EDTA disodium salt (Fison Lab. reagent)
in 1L distilled water. pH 7.6 and pH 8.6, pH adjusted with 10M NaOH.

7. Polyethylene Glycol (PEG)

MW 6000 Daltons, Hopkins and Williams Ltd.

a - stock solution 20% W/V:

This was made up from 20g polyethylene glycol in 100ml of working BBS.

b - Working solution of PEG:

12% PEG in working BBS and 60mM EDTA pH 7.5 made up from:

6ml 20% W/V PEG in BBS
3ml 0.2M EDTA solution pH 7.6
1 ml working BBS

c - Washing solution of PEG:

2% PEG in 10mM EDTA pH 7.6 and BBS made up from:

5ml stock PEG in BBS
2.5ml 0.2M EDTA solution pH 7.6
42.5ml BBS

8. Agarose (Indubiose A37) IBF Ltd.

Methods

The assay involved precipitation of soluble immune complexes from serum by the addition of polyethylene glycol (PEG). The amount of C1q, IgG and IgM in the precipitate was measured by single radial immunodiffusion (SRID), and presence or absence of IgA by double diffusion in agarose gel. Values were compared with those obtained in this test from pooled and single (normal) human sera from healthy individuals.

PEG precipitation:

0.1ml of solution of 12% PEG in BBS containing 60mM EDTA pH 7.6, was added to 0.5ml of the test serum. The solutions were mixed thoroughly, and left overnight at +4°C. Solutions were mixed again before centrifuging at 1000x g for 20 minutes (+4°C). The supernatants were decanted by pouring and kept at +4°C. The precipitates were washed once with 2ml of 2% PEG in BBS containing 10mM EDTA pH 7.6, mixed, and then spun as above (1000 x g; 20 minutes; +4°C). The "wash" was discarded and the pellet redissolved in 0.5ml BBS. This was then well mixed, and left for at least 30 minutes at room temperature.

Mancini Gel Preparation

(For the estimation of C1q, IgM and IgG)

Each Mancini gel plate contains a total volume of 25 ml including the antiserum. The Mancini plate was prepared as the following example for 10% anti-C1q antibody:

250mg Agarose (indubiose A37, IBF Ltd.)

5 ml 0.2 M EDTA pH 8.6

17.5 ml working BBS

2.5 ml anti-C1q

The agarose, EDTA and BBS were gently heated until the agarose dissolved and melted. This was then cooled to 50°C and the antibody added and mixed. The solution was then injected between 2 glass plates (250mm x 150mm), held 1mm apart by a rubber separator, where it rapidly cooled - as shown by clouding of the gel. The plate was slid off and 4mm diameter holes were punched in the gel and the circles of the agarose were removed by suction. The distance between the centres of the wells in each row and column was 15mm. This allowed a total of 66 wells per plate.

Single Radial Immunodiffusion (SRID)

10µl of each sample (the PEG pellet re-suspended in BBS) was put in each well. In addition, 10µl of pooled or single, normal human sera (suitably diluted in BBS) were added for controls. The plates were incubated overnight at + 37°C, and precipitin ring diameters measured the following day to the nearest 0.1mm. The amounts of antigen were calculated from the standards included in each plate.

Double Diffusion:

A petri dish containing a 1mm thick 1% agarose gel was prepared by pouring the heated agarose solution into a horizontally placed dish. When cool, a hexagonal pattern of 4mm diameter wells was then punched in it. 2µl anti-IgA antiserum was placed in the central well. 10µl of the sera under test including one normal serum sample acting as control,

was put in the wells around it. The plate was incubated at +37°C overnight, and examined for the presence or absence of a precipitin arc the following day.

Calculation of results

At equilibrium, when antigen diffusion out of the 4mm diameter well has ceased, the area within the "precipitin ring" formed is proportional to the amount of antigen originally present.

Measurement of the diameter of this ring gives an expression of the amount of antigen in the sample tested (Mancini et al, 1965). For example:

Let the mean of the diameter of 3 normal serum standards = S(mm) and the unknown diameter = U(mm)

Knowing that (a) the diameter of the well = d = 4(mm)
and (b) the dilution factor D of the normal serum for each plate was as below

Plate	Assessment of:	NHS dilution	D
Anti - C1q	precipitate	undiluted NHS	1
Anti - C1q	supernatant	5/6 dilution	5/6
Anti - G	precipitate	1/50 dilution	1/50
Anti - M	precipitate	1/5 dilution	1/5

Then, since area of precipitation = area of outer circle-area of inner circle

$$\text{Standard area of precipitation} = A=\pi\left(\frac{S}{2}\right)^2 - \pi\left(\frac{d}{2}\right)^2$$

$$A=\pi\left(\frac{S^2}{4} - \frac{4^2}{4}\right)$$

$$\text{Unknown area of precipitation} = X = \pi\left(\frac{U}{2}\right)^2 - \pi\left(\frac{d}{2}\right)^2$$

$$X = \pi\left(\frac{U^2}{4} - \frac{4^2}{4}\right)$$

And so, the ratio $\frac{X}{A}$ represents an expression for the relative amount of antigen in PEG pellet compared with "standard" normal human serum.

$$\frac{X}{A} = \frac{\pi\left(\frac{U^2}{4} - \frac{4^2}{4}\right)}{\pi\left(\frac{S^2}{4} - \frac{4^2}{4}\right)}$$

$$\frac{X}{A} = \frac{(U^2 - 4^2)}{(S^2 - 4^2)}$$

since A = 100% $\qquad X = \frac{(U^2 - 4^2)}{(S^2 - 4^2)} \times 100\%$

and allowing for the dilution factor D:

$$X = \frac{(U^2 - 4^2)}{(S^2 - 4^2)} \times D \times 100\%$$

A computer program was constructed to calculate this. By measuring the PEG precipitates of 80 normal sera - the mean and standard deviation (S.D.) of this control group were calculated, and an upper limit of normal designated as mean + 2 S.D. values above these limits for any component (C1q, IgG, IgM) indicated positive IC. The parameters of normal sera are shown in Tables 2.1 and 2.2.

Table 2.1 PEG PRECIPITATION

Here are some fairly arbitrary values to represent those given by normal people:

UPPER LIMITS OF NORMAL = mean + 2 SD's

1) In terms of %

	mean	SD	ULN
C1q	22	15	52
IgG	0.39	0.15	0.69
IgM	2.22	1.75	5.72
IgA	- lower limit of sensitivity - 0.18%		

2) In terms of concentration

C1q	44µg	30µg	104µg	all from 1 ml
IgG	39µg	15µg	69µg	
IgM	44µg	35µg	114µg	
IgA	- lower limit of sensitivity - 11.2 - 32µg			

Table 2.2 SERUM IMMUNOGLOBULINS AND COMPLEMENT COMPONENTS

SERUM IMMUNOGLOBULINS

	In terms of %			
	mean	SD	mean ± SD	mean ± 2SD
IgG	86	15	71 - 101	
IgM	92	26	66 - 118	
IgA	146	52	94 - 198 (!)	
COMPLEMENT COMPONENTS etc				
C1q	110	9	101 - 119	92 - 128
C3	101	11	90 - 112	79 - 123
CH50	99	9	90 - 108	81 - 117

For these purposes serum levels of components taken to be as follows:

C1q - 200µg/ml
IgG - 10mg/ml
IgM - 2mg/ml
IgA - 1.4 - 4.0mg/ml

The sera that these results represent are a mixture (approx = numbers) of aliquots of various different pools of ANCs and various serum samples from laboratory 'normals' at the most 3 samples from any one person.

Estimation of C3

Serum C3 was measured by single radial immunodiffusion, using the technique described previously, but with the following differences:

The distance between the centres of the wells in each row and column of each Mancini plate containing anti-C3 was 20 mm.

5 µl of each studied serum was put in each well, and 5 µl of normal human serum was added to the last 3 wells as a control.

Estimation of CH50

The method used for the estimation of CH50 was a modification of the method described by Nydegger et al (1972).

Solid Phase Radioimmunoassay for Cross-reaction of Complexes

The principle of this technique is that tubes are coated with complexes which have been precipitated from serum with polyethylene glycol. The complexes are dissociated in the tubes in glycine/HCl. In preliminary experiments (Dambuyant et al, 1979) it was found that coating in acid is quite satisfactory. After the coated tubes have been washed radio-labelled polyethylene glycol pellets from other sera are added to the tubes, again in acid. The tubes were then neutralised, so that the antibody can re-combine with antigen. If there is cross-reaction between the coating complexes and the labelled ones, some of the label will be attached to the complex on the wall, labelled antigen binding to coating antibody, and vice versa. To reduce nonspecific sticking of the labelled complex to the wall, and to block the effects of rheumatoid factors in the mixture, the reaction is done in an excess of horse serum. The amount which produced maximal blockade of rheumatoid factor, and also saturated any nonspecific protein binding sites on the wall of the tube, was found by experiment. I tried to add even more horse serum to reduce the background further, but 10 microlitres was found to produce the maximal effect.

In the original description of the method by Dambuyant et al (1978, 1979) the method was validated by the use of synthetic BSA/anti-BSA complexes, and human C3/anti-C3 complexes. These demonstrated that there was antigenic specificity in the radioimmunoassay and that the amount of binding to the tube was dependent on the antigen-antibody ratios in the two complexes. Thus if antigen excess complexes were used to coat it was difficult to get such complexes to interact with the labelled complex, due to lack of coating antibody sites. The reverse would occur if antibody excess complexes were used to coat, when only antigen excess complexes would stick sufficiently for statistically significant amounts to be detected. It is for this reason

that some complexes do not easily coat tubes for the adherence of the same when radiolabelled complexes ("self cross-reaction"). Thus patients sometimes appear not to cross-react with themselves, but can do with others sharing the same antigenic specificity.

Solid Phase Radioimmunoassay (RIA) for Cross-Reaction of Complexes

Materials

1. Sera

Sera from patients with polycystic kidney, SLE, Tb, sarcoidosis and pericarditis and from patients with various immune complex diseases and their relatives including family contacts were studied. These sera were provided by University College Hospital, St. Mary's Hospital, The Middlesex Hospital, Brompton Hospital and the Freeman Hospital or Royal Victoria Infirmary, Newcastle-upon-Tyne. Standard sera were taken from laboratory workers. After clotting, sera were stored in 5 ml aliquots at -70°C till used.

2. PEG: Prepared as described previously.

3. BBS: Prepared as described previously.

4. 0.1 M glycine/HCl buffer pH 3.1

0.1 M glycine/HCl buffer made up from:

856 ml solution A + 144 ml solution B + 0.1 ml phenol red/distilled water solution. The pH was adjusted to 3.1 with 10 N sodium hydroxide.

Solution A: 0.1M glycine in 0.1 N sodium chloride made up from:

7.5 g glycine (Hopkin and Williams Ltd.)
5.86 g sodium chloride (Fisons Ltd.) in 1 litre distilled water.

Solution B: 0.1 N hydrochloric acid made up from:

8.6 ml hydrochloric acid, HCl (BDH Chemicals Ltd.) in 1 litre distilled water.

Phenol red and sodium hydroxide were purchased from BDH Limited.

10 N sodium hydroxide: made up from:

400 g sodium hydroxide NaOH in 1 litre distilled water.

5. 0.2 M Phosphate Buffer pH 7.2

made up from: 280 ml 0.2 M NaH_2PO_4
720 ml 0.2 M $Na_2H\ PO_4$
pH to be adjusted to 7.2 with 0.1 N hydrochloric acid.

0.2 M NaH_2PO_4: made up from:

31.2 g $NaH_2PO_4\ 2H_2O$ sodium dihydrogen orthophosphate hydrated (Hopkins and Williams) in 1 litre distilled water.

0.2 M $Na_2H\ PO_4$: made up from:

28.39 g $Na_2H\ PO_4$ disodium hydrogen orthrophsphate (BDH Chemicals Ltd.) in 1 litre distilled water.

6. 0.5 M phosphate buffer pH 7.5

made up from: 280 ml 0.5 M NaH_2PO_4
720 ml 0.5 M $Na_2H\ PO_4$
pH to be adjusted to 7.5

7. Phosphate buffered saline (PBS)

40 ml 0.2 M phosphate pH 7.2 + 17 g sodium chloride in 2 litres of distilled water

8. Neutralising solution (0.1 M phosphate/0.5 N NaOH solution)

equivalent volumes of 0.2 M phosphate buffer pH 7.5 and N NaOH

9. Chloramine T solution:

made up just before use from:

50 mg chloramine T (BDH Chemical Ltd.)
2 ml 0.5 M phosphate pH 7.5

10. Sodium metabisulphite solution

made up just before use from:

40 mg sodium Metabisulphite (BDH Chemical Ltd.)
2 ml 0.5 M phosphate pH 7.5

11. ^{125}Iodine

($Na^{125}I$ Radiochemical Centre, Amersham)
The $Na^{125}I$ solution was diluted with PBS to give 1.8×10^7 count per minute per 10 µl.

12. Normal Equine Serum (NES)

(Wellcome Reagents Ltd.)

13. Autoanalyzer cup

(Conical Cup LS.127 Altec Ltd.)

Methods

1. Isolation and estimation of immune complexes (IC).

This was carried out according to the method described previously.

2. Labelling of complexes

Complexes were labelled according to the method of Dambuyant et al (1979) a modification of the procedure worked out by Hunter (1973). 100 µl of the IC solution was mixed with 10 µl of ^{125}I(1.8×10^7cpm) 20 µl chloramine T (BDH Chemicals Ltd.) in 0.5 M phosphate buffer pH 7.5 was added, and after 45 seconds, the reaction was stopped by adding 100 µl sodium metabisulphite in 0.5 M phosphate buffer pH 7.5.

0.4 ml PBS was added, and the solution dialysed overnight against 2L PBS (the solution was changed once in this time).

The dilution of the radioactive solution was adjusted with PBS, so that 20,000-30,000 cpm of the labelled complex solution could be used for each tube in the cross reaction assay.

3. Cross reaction assay

10 µl volumes of the IC solution was coated onto plastic autoanalyzer cups in 1 ml 0.1 M Gly-HCl pH 3.1 for 2 hours at room temperature. In addition to the rows of cups being coated with ICs, 2 to 3 rows of empty cups (not coated with ICs) were included as controls, and were treated in all other respects like the coated tubes. After 3 washes with 0.9% NaCl solution, 1 ml of 1% normal horse serum in 0.1 M gly-HCl pH 3.1 was added to each cup, and after 5 minutes, the ^{125}I labelled complexes were added in columns. Neutralization to pH 7.5 by the addition of

$NaOH/Na_2H\ PO_4$ buffer was carried out after a further 20 minutes.

The volume of $NaOH/Na_2H\ PO_4$ required to do this was previously determined by titration; the end point shown by change in the indicator colour (yellow to pink).

The cups were left overnight at room temperature and after removal of unbound material by suction, and 3 saline washes, the cups were counted in a Nuclear Enterprises 8311 gamma spectrometer to determine the amount of ^{125}I-labelled complex bound to the coated tubes.

Calculation and presentation of results:

Percentage binding was calculated using a computer program which worked it out as shown below:

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

A 2-dimensional matrix diagram was constructed in which the horizontal rows related to each complex used to coat a cup, and the vertical columns related to each labelled complex added to the cups. Values were considered not significant if they were less than 2 S.D. greater than the binding to the control cup. Only values significant and greater then 0.5% were considered as positive binding (Burton-Kee et al, 1979).

Cross Reaction by the Delayed Technique

This technique was in the process of development towards the end of my studies, but Mrs Zewdie who had developed it in the laboratory had got it to a stage where it might be useful to try in the pericarditis patients. The technique overcomes some of the troubles found when studying IgM complexes, as it does not require the acid conditions which are very destructive to IgM. The solid phase assay was thus not a good one in the pericarditis patients, many of whom had complexes in which the immunoglobulin was largely, or wholly IgM.

This method uses to a advantage that such sera contain very little polyethylene glycol precipitable material. If a mixture of an IgG antibody and a serum containing immune complexes of IgM is made, as time passes the repeated dissociation and re-association of antibody will result in a new equilibrium in which the complexes will then contain a mixture of the IgG and IgM antibodies combined with the antigen. Obviously if the antibody does not cross-react with complexed antigen this will not occur. After a time, found experimentally to be more than four days, the complexes are precipitated with polyethylene glycol, and the amount of precipitated IgG is then found by single radial diffusion. In preliminary work with this technique mixtures were precipitated at intervals of up to 10 days, and the amount of bound IgG measured. With the model complexes used it was only after about four days that the amount of bound IgG had reached a plateau.

Preliminary work aslo showed that using the serum of a child with IgM complexes of rubella viral antigen, the haemagglutinin, positive binding of an IgG rubella antibody was found after the mixture had been left for four days.

In order that no other interaction involving complement activation could occur, and also to preserve the sterility of the samples, all the incubations were done in 10mM EDTA at 4C.

The ratio of 80 to 20 for serum containing complexes and antiserum respectively was based on two factors. First was the need to get as much immune complex as possible into the system to increase the sensitivity, and the second was the attempt to mimic the clinical situation. The transfusion of a unit of blood into an average adult could be approximated by this ratio, and gave as much space to the complex containing serum as possible. Using this ratio in our preliminary experiments seemed to work better than equal amounts of the two sera so was used in all subsequent experiments.

Cross-Reaction by the Delayed Technique

This method shows the cross-reaction between the IgG of the normal and the circulating immune complexes of the patients.

Materials

1. Patients' sera. After clotting, sera were stored in 5 ml aliquots at - 70°C till used.
2. Donor's sera. As above.
3. Anti IgG - Mancini gel preparation.
4. 60 mM EDTA pH 7.6.
5. BBS.
6. Polystyrene tubes (LP3 Luckham Limited, Sussex)
7. Working PEG.
8. Washing PEG.

Methods

20 µl of the donor serum was mixed with 80 µl of the patient's serum. 20 µl of 60 mM EDTA pH 7.6 was added to each tube and the mixture was kept in +4°C for six days. 100 µl of each of the patient's and donor's samples unmixed were also studied in the same way. On the sixth day 20 µl of the 12% working PEG solution was added. After mixing thoroughly, it was left for 18 hours at +4°C.

Solutions were mixed again before centrifuging at 1000 x g for 20 minutes (+4°C). The supernatants were discarded and the precipitates were washed once with 1 ml of washing PEG (2%), and then spun as above (1000 x g; 20 minutes, +4°C). The wash was discarded and the pellet redissolved in 100 µl of BBS.

The IgG fraction that had been precipitated in each pellet was estimated by SRID.

The results of the mixture were considered positive when the percentage of IgG precipitated in the mixture was greater than the percentage of IgG expected.

Percentage of IgG expected =

0.8(% patient's IgG precipitated alone) + 0.2 (% donor's IgG precipitated alone).

Rheumatoid Factor Binding Assay

I attempted to make an assay for cross-reaction based on the technique for detecting complexes described by Barratt and Naish (1979). If an IgG antibody is added to a complex containing serum, and the IgG becomes bound in the complex, more rheumatoid factor could be bound on the complex than would be the case if the IgG antibody were not added. The technique would also probably require that the complexes were in antigen excess for the IgG to be bound, although the delayed technique described overcomes this by waiting for a new equilibrium to occur. In the laboratory there was a large volume of serum from a patient with a very large amount of rheumatoid factor, and considerable quantities of it were available. It was decided then to use this serum as a source of a standard rheumatoid factor which could be labelled and used in this test. The serum contained about 50 grams per litre of IgG rheumatoid factor, with smaller amounts of IgM and IgA rheumatoid factor. The IgG was chosen for this because such small amounts of IgG are precipitated with polyethylene glycol compared with IgM and the test should therefore be more sensitive. In retrospect this was probably a mistake, since the divalency of IgG prevents any substantial increase in the binding to complexed as opposed to unpolymerised IgG. By contrast, with IgM rheumatoid factor the valency of binding can rise from unity with native IgG to a maximum of five when bound to complexed IgG.

Rheumatoid Factor Binding Assay

Materials

1. Sera: Sera from patients with pericarditis were provided by Freeman Hospital or Royal Victoria Infirmary, Newcastle-upon-Tyne. Other studied sera were taken from patients with various IC diseases and their family contacts.

2. BBS, PEG, PBS, iodine, chloramine T and sodium metabisulphite

   As described previously.

3. Isolation of rheumatoid factor used:

   a) Starting plasma: Removed by plasmapheresis from a patient (SH) with severe cutaneous vasculitis. Immune complexes were detected in the plasma by the PEG precipitation and a SCAT (sheep cell agglutination test) revealed the presence of large amounts of rheumatoid factor.

   b) Preparation of IgG-rich RF(GRF): A solution previously prepared by a standard Na_2SO_4 precipitation method was stored in aliquots at $-70^{\circ}C$.

4. Analysis of RF solutions obtained:

   Protein estimation using the biuret method: The biuret method is a quantitative colorimetric method of assaying protein solutions (Kabat and Mayer, 1961).

   a) Bovine serum albumin (BSA): A standard protein solution was made up containing 5 mg/ml BSA in BBS, and various dilutions were made from this stock

   b) Biuret reagent contained: 1g Sodium-potassium tartrate, 3g Hydrated copper (II) sulphate, 5g Potassium iodide } in 1l carbonate free 0.2N sodium hydroxide.

c) RF solutions: were suitably diluted to a final volume of 1 ml with 0.5 M $NaHCO_3$.

Method

To 1 ml of various dilutions of the "unknown" protein solution in 0.5M $NaHCO_3$ was added 1.5 ml biuret reagent. The solutions were mixed and incubated at + 37°C for 30 minutes. The OD_{540} was then read on a Gilford 4N spectrometer. The amount of protein in the RF solution was calculated from the calibration curve constructed by measuring the OD_{540} of various dilutions of the standard protein in (BSA) solution. The GRF stock was found to contain 3.24 mg/ml.

5. Labelling of rheumatoid factor:

Using the method of Dambuyant et al (1979), but with the following modifications:

IgG RF: 10 µl IgG RF : BBS 10:100 diluted stock
+10 µl ^{125}I (1.8×10^7 counts/min) made up to 330 µl
+20 µl Chloramine T with PBS after dialysis
+100 µl Sodium metabisulphite

Labelled RF's were stored in small aliquots at -20°C and thawed just before use.

Rheumatoid factor binding assay method:

20 µl of the donor's serum was mixed with 80 µl of the patient's serum in duplicate polystyrene tubes (LP3). The contents were mixed and left at +37°C for 1 hour. 100 µl of each of the patient and donor samples unmixed were also studied and treated in the same way.

20 µl of 12% PEG and 10 µl ^{125}I-RF were then added to each tube and the contents mixed and left overnight at +4°C. This was then mixed, spun at 1000g, +4°C for 20 minutes, and the supernatant discarded. The precipitates were washed once with 1 ml of 2% PEG in BBS containing 10 mM EDTA pH 7.6 and the contents mixed, then spun as above, and the

supernatants discarded. The precipitate was re-suspended in 0.5 ml BBS and counted in a gamma spectrometer (Nuclear Enterprises No. 8311) to determine the fraction of the rheumatoid factor which had bound to the complex. The binding in the mixture was then expressed as positive when it was greater than the expected value, that which would be obtained with the appropriate amounts of the individual sera unmixed. Counts bound were expressed as a percentage of total counts added to the tubes.

Calculation of results:

Positive binding in the mixture would be defined as a significant increase over the expected value.

Expected value = 0.8 (percent ^{125}I-RF bound to recipient's serum) +
+0.2 (percent ^{125}I-RF bound to donor's serum)

N.B. The preparation of rheumatoid factor and the estimation of its protein content were done by Mrs J.B.Kee.

Plasma Treatment

Plasma treatment was performed by transfusing one unit or more of plasma from a suitable donor to the patient. Suitability was defined as a healthy donor whose serum cross-reacted with recipient's immune complex by RIA. The cross-reaction between the donor and the patient by the delayed technique was also studied.

Materials and method:

Patient: Patients with various immune complex diseases or with infectious diseases were transfused with plasma from their close relatives (including spouse).

Donors: Suitability was assessed by RIA technique using serum sample taken from the family of patients. All donors were Australia Antigen negative

Follow up: 1. Clinical observation before and after plasma infusion.

2. Immune complex measurement from serum both pre- and post-infusion.

N.B. Plasma infusion was followed by immunoglobulin injections in some patients and two patients were treated with plasmapheresis (see Table 2.3).

Table 2.3 LIST OF THE TREATED PATIENTS

Patient's name	Sex	Disease	Donor
SG	F	EM	son
TH	M	EM	father
MO	F	EM	son
JS	M	EM	FFP
FA	F	SLE	(PP)
FM	F	SLE	brother
WM	F	SLE	(PP)
FB	M	WG, FGN	wife
IJ	F	MCTD, cutaneous vasculitis	FFP
RJ	M	Rh. Arth., renal vasculitis	brother
FL	F	ITP, cutaneous vasculitis	sons
RM	M	Allergic vasculitis	wife
FP	F	Cutaneous vasculitis	brother & sister
KR	F	PAN	husband
HZ	M	Active hepatitis & renal & cutaneous vasculitis	son & daughter
DB	F	FPGN	husband
KG	F	FGN	sister
SAH	M	MCGN	father
LP	M	MCGN	daughter
WW	M	FPGN	daughter
JG	F	Chronic pericarditis	husband
SH	F	Chronic pericarditis	husband
MS	F	Chronic pericarditis	immunoglobulin
JW	F	Chronic pericarditis	husband

Key: EM = Erythema multiforme. SLE = Systemic lupus erythematosus.
WG = Wegener's granulomatosis. FGN = Focal glomerulonephritis.
MCTD = Mixed connective tissue disease. Rh.Arth. = Rheumatoid arthritis.
ITP = Idiopathic thrombocytopenic purpura. PAN = Polyarteritis nodosa.
FPGN = Focal proliferative GN. MCGN = Mesangiocapillary GN.
FFP = Fresh frozen plasma. PP = Plasmapheresis.

CHAPTER THREE
RESULTS

Composition of Immune Complexes in Infectious Diseases

For comparison, sera from patients with pericarditis and with other diseases were analysed for immune complexes, (isolation and estimation of immune complexes have been described previously):

a. 353 samples from 218 patients with percarditis were examined for immune complexes in the period January 1978 to March 1980. They were obtained from the Newcastle area.

b. 230 sera taken from patients with various diseases were analysed. Table 3.1 below shows the range of diseases which these sera represent.

c. 13 sera from patients with systemic lupus erythematosus, 60 sera from patients with subacute bacterial endocarditis and 29 sera from patients with ankylosing spondylitis were also studied and compared with the above sera.

The composition of immune complexes in sera studied are shown in Table 3.2.

Table 3.1 COMPARISON OF 230 SERA USED IN STUDY

Diagnosis	No. of serum samples	Diagnosis	No. of serum samples
Vasculitis	13	PUO	3
Renal Failure	21	Dermatitis Herpetiformis	12
SBE	43	Erythema Multiforme	3
PAN	7	Septicaemia	7
Glomerulonephritis	31	SLE	9
Ankylosing spondylitis	17	RA	3
Pelvic inflammatory disease	8	Dresslers	2
Endocarditis	4	Urticaria	3
Cervical cancer	2	Cryoglobulinaemia	4
Myeloma/lymphoma	3	Rheumatic fever	2
		Others	33

Table 3.2 COMPOSITION OF IMMUNE COMPLEXES IN SERA STUDIED

Complex contains	Pericarditis	All other sera	SLE	SBE	AS
C1q, IgM, IgG, IgA +/- other components	58.7 (+)	68.3	53.9	72.7	75.0
	61.2 (+)	50.3	69.2	61.4	25.0
	51.3 (*)	71.4	92.3	75.0	87.5
	52.9 (NS)	49.7	61.5	56.8	31.3
IgM but not IgG	28.1 (*)	11.8	0	6.8	0
IgG but not IgM	18.2 (*)	31.7	32.1	20.5	62.5
IgG and IgM	33.1 (±)	39.8	69.2	54.6	25.0
IgM only	19.8 (*)	7.5	0	6.8	0
IgG only	10.8 (*)	19.9	7.7	4.6	50.0
IgA only	15.7 (±)	9.3	7.7	9.1	0
Number of immune complex positive sera analysed	242	161	13	44	16

KEY: SBE = subacute bacterial endocarditis
SLE = systemic lupus erythematosus
AS = ankylosing spondylitis
Values represent proportions (%) of immune complex positive sera

Statistically significant difference using Student's t-test (between "pericarditis" and "all other sera")
+ $p < 0.01$; ± $p < 0.05$; * $p < 0.001$; NS = not significant

The proportion of sera which were positive for immune complexes in each disease group is shown in Table 3.3.

TABLE 3.3 PROPORTION OF SERA CONTAINING IMMUNE COMPLEXES:

Diagnosis	total no. of sera tested	No. of IC + ve sera	% sera + ve for IC
Pericarditis	353	242	68.5
All other sera	230	161	70
SBE	60	44	73
SLE	13	13	100
Ankylosing spondylitis	29	16	55.1

The following observations can be summarised from the previous data:

1. There is a significant increase in the proportion of IgM containing complexes in the pericarditis group ($p < 0.01$). This is probably due to the increase in "IgM-only" complexes ($p < 0.001$).
2. The proportion of IgG complexes in the pericarditis group is less than the other sera studied ($p < 0.001$). This may be due to the drop in "IgG-only" complexes.
3. There is a significant increase in "IgA-only" complexes in pericarditis sera ($p < 0.01$).
4. The amount of C1q in pericarditis complexes is significantly less than in other studied sera ($p < 0.01$).

N.B. - C1q levels in circulating complexes in patients with SLE are lower than the other sera studied, because they represent the fraction of normal C1q levels precipitated in SLE. Where total C1q levels are low, the fraction of patient C1q which is complexed is very high.

- The above data were taken from our laboratory records and the studies were done by J. Burton-Kee and S.G. Johannes.

Cross-Reaction by Radioimmunoassay

The aim of this assay is to provide further evidence of the presence of a common causal agent(s) in a group of patients by showing cross-reaction between their immune complexes.

Immune complexes in sera from patients with polycystic kidney, systemic lupus erythematosus, tuberculosis, sarcoidosis and dermatomyositis were precipitated with polyethylene glycol (PEG). The antigenic cross-reactions between samples were studied by the method of Dambuyant et al (1979). The technique used has been described earlier. Statistically significant values greater than 0.5% are shown by black squares.

a. Experiment with sera of polycystic kidney patients:

5 Samples from patients with polycystic kidney were studied. The dissolved pellets were used to coat a series of cups. The same pellets after iodination were added to test the cross-reactivity between each other. A two-dimensional matrix was constructed in which the horizontal rows related to each complex used to coat a cup, and the vertical columns related to each labelled complex added to the cups. The cross-reaction results are shown in Fig. 3.1. I have found greater than 0.5% binding in 9 of the 25 cross-reactions performed (36% of all reactions).

b. Experiment with sera of patients with systemic lupus erythematosus:

The cross-reactions found within the whole systemic lupus erythematosus group are shown in Fig. 3.2. I have found greater than 0.5% binding in 63 of the 175 cross-reactions performed (36% of all reactions).

c. Experiment with sera of patients with tuberculosis:

Cross-reactions of 24 labelled complexes on 29 coated tubes were studied. I have found greater than 0.5% binding in 113 of the 696 cross-reactions performed (16.2% of all reactions). The cross-reaction results are shown in Fig. 3.3.

d. Experiment with sera of patients with sarcoidosis:

The solid phase cross-reactions technique was applied to 13 sera which came from sarcoidosis patients. The cross-reactions results between the complexes of these patients are shown in Fig. 3.4. I have found greater than 0.5% binding in 37 of the 169 cross-reactions performed (21.8% of all reactions).

e. Tuberculosis and Sarcoidosis:

Some cross-reactions between complexes of tuberculosis and sarcoidosis patients were positive and are shown in Fig. 3.5. These two groups seem to share antigenic determinants or antibody specificity or a mixture of both (Dambuyant et al, 1979).

f. Experiment with sera of patients with dermatomyositis:

Sera from 3 patients with dermatomyositis were studied and the cross-reaction results between their complexes were negative. Results from previous studies with some other disease groups are shown for comparison in Fig. 3.6 (Dambuyant et al, 1979; Burton-Kee et al, 1979).

Values above 2% were considered to be positive for the bullous pemphigoid and hypertension groups. The percentage of positive reactions within various disease groups are shown for comparison in Fig. 3.7.

N.B. - The cross-reaction results between the donors and the recipients by this method are shown individually in the patient studies.

- The cross-reactions between complexes of the pericarditis patients were done by S.G. Johannes.

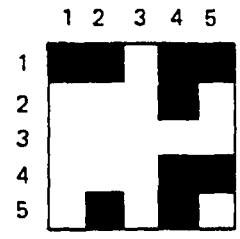


Fig 3.1
Cross-reactions between complexes of polycystic kidney patients

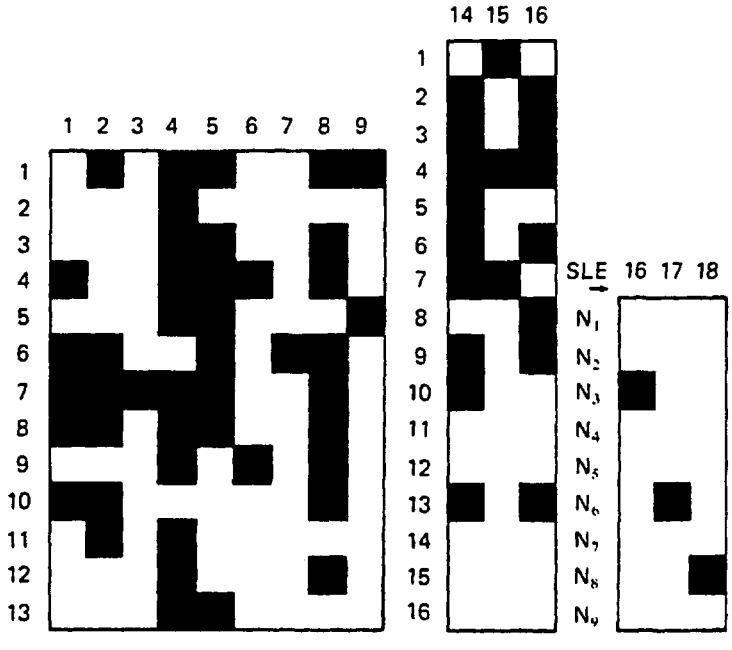


Fig 3.2
Cross-reactions within SLE disease group

N = normal

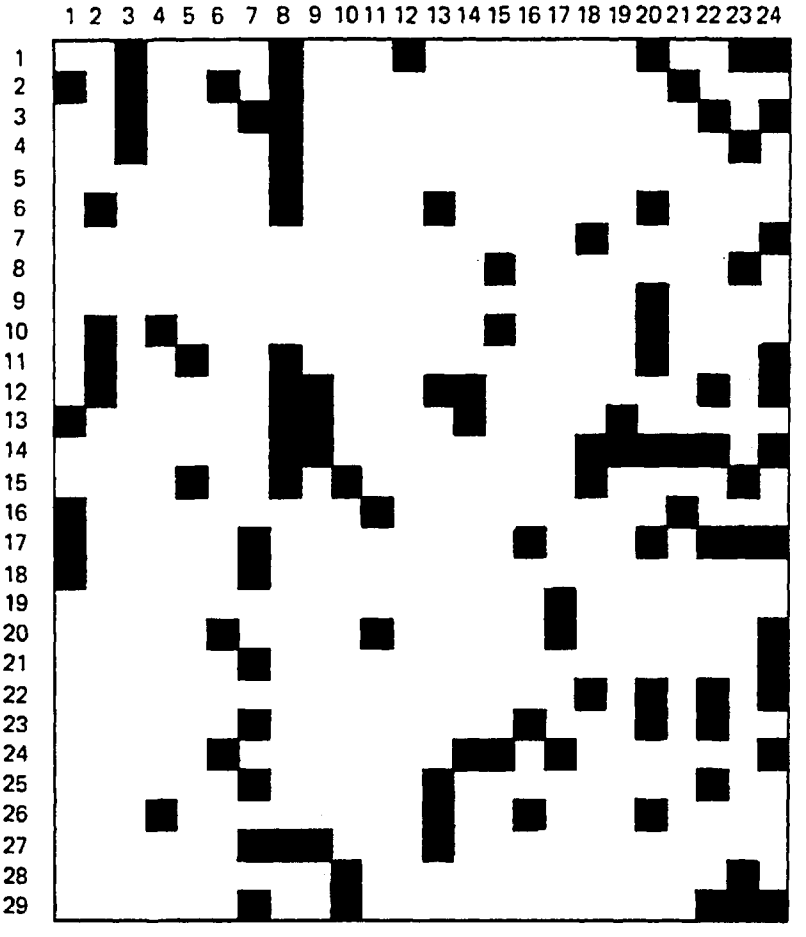


Fig 3.3
Cross-reactions between complexes of tuberculosis patients

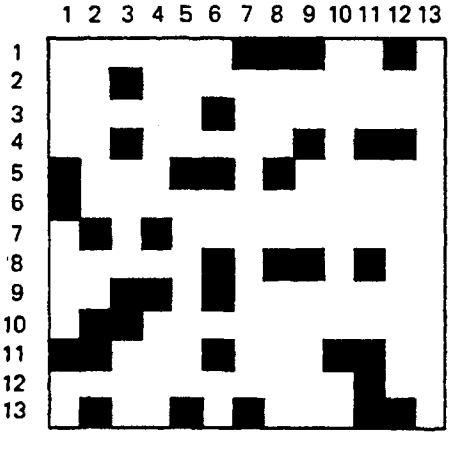


Fig 3.4
Cross-reactions between complexes of sarcoidosis (Sa) patients

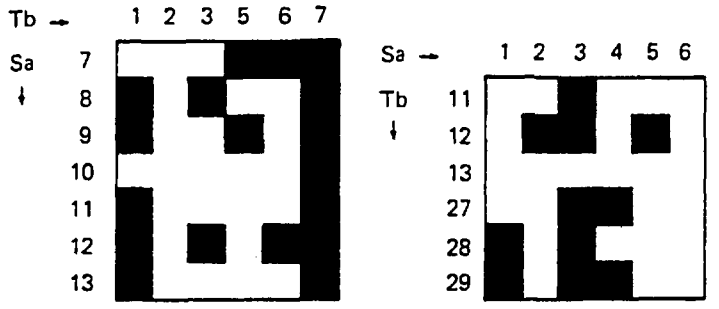


Fig 3.5
Cross-reactions between complexes of tuberculosis and sarcoidosis patients

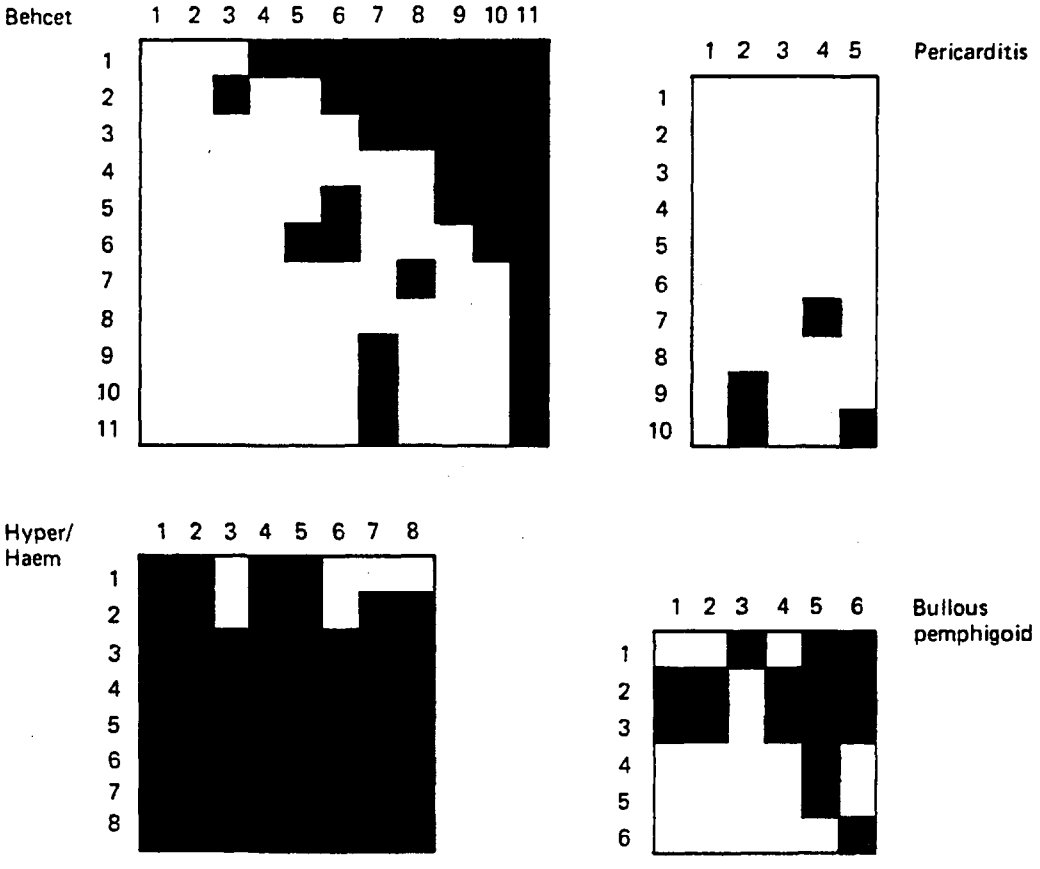


Fig 3.6
Cross-reactions within four disease groups. Behcet = patients with Behcet's syndrome Pericarditis = patients with pericarditis. Hyper/Haem = patients with hypertension and haematuria. Bullous pemphigoid = patients with Bullous pemphigoid.

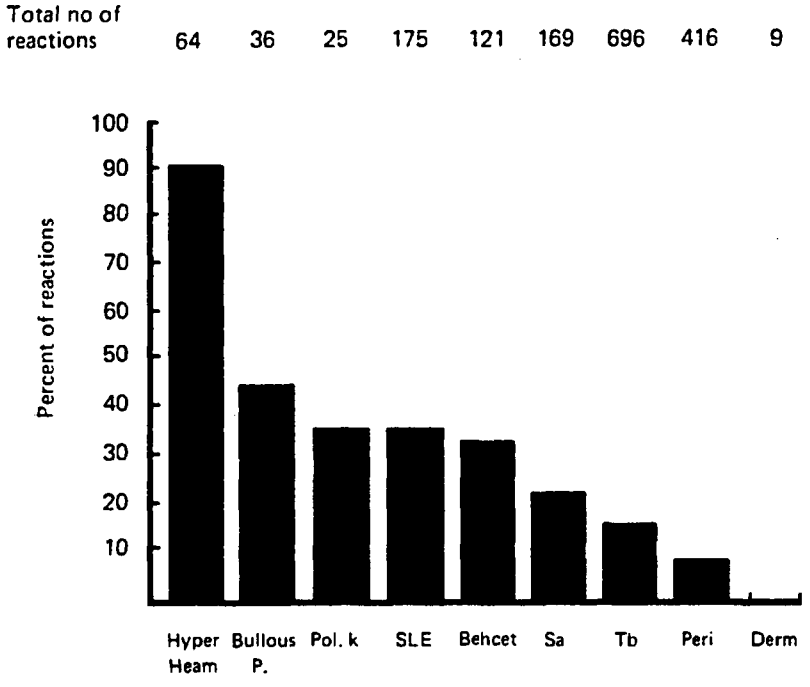


Fig 3.7
Percent of positive reactions in various disease groups

Key.
1. Hyper/Haem – Hypertension/Haematuria
2. Bullous P. – Bullous pemphigoid
3. Pol. k – Polycystic kidney
4. Sa – Sarcoidosis
5. Tb – Tuberculosis
6. Peri – Pericarditis
7. Derm – Dermatomyositis

Cross-Reaction by the Delayed Technique

This technique has been developed recently in our laboratory, and we started to use it to study the cross-reaction between the patient's immune complexes and the donor's sera.

This method is more specific for IgG complexes, and some positive results were correlated with RIA positive results in certain patients e.g. F.B., K.R., F.P. etc.

Rheumatoid Factor Binding Assay

Using the IgG rheumatoid factor of a patient with rheumatoid arthritis with a high titre of rheumatoid factor, it has been possible to measure the ability of circulating complexes to bind this rheumatoid factor.

This method was used to study the ability of donor antibody to react with patient complexes.

a. Donor-recipient sera interaction:

1:4 mixture of donor/recipient sera were studied. The donors were close relatives (including family contacts). The ability of their serum samples to react with the patient's circulating complexes was studied in an attempt to see if it was possible to know which donor plasma was suitable for infusion. The results are shown in Table 3.4. The positive bindings are underlined.

b. Experiment with sera of pericarditis patients:

1:4 mixture of normal-pericarditis sera were studied as above. The results are shown in Tables 3.5, 3.6 and 3.7.

Table 3.4 DONOR-RECIPIENT INTERACTION RESULTS.

1. Patient LP	27	21. Patient RJ	9.8
2. Donor MP	32	22. Donor WJ	15
3. Mixture	18	23. Mixture	9.7
4. Expected	28	24. Expected	10.8
5. Patient FP	27	25. Patient WI	30
6. Donor AP	31	26. Donor JI	29.2
7. Mixture	29.8	27. Mixture	23
8. Expected	27.8	28. Expected	29.8
9. Patient WW	30.5	29. Patient KR	7.7
10. Donor JW	22	30. Donor JR	22
11. Mixture	15	31. Mixture	15
12. Expected	28.2	32. Expected	10.2
13. Patient SAH	23	33. Patient BA	11.3
14. Donor SA	18	34. Donor PA	11.4
15. Mixture	20.5	35. Mixture	15
16. Expected	22	36. Expected	11.3
17. Patient DB	10	37. Patient KG	15
18. Donor NB	19.3	38. Donor FP	18.5
19. Mixture	5.8	39. Mixture	17
20. Expected	11.9	40. Expected	15.7

N.B. (1) Values represent percentage ^{125}I-RF bound to each sample studied.

(2) Expected values =
0.8(% ^{125}I-RF bound to recipient's serum) + 0.2(% ^{125}I-RF bound to donor's serum)

(3) Positive bindings are underlined.

Table 3.5 PERICARDITIS MIXTURES ASSAY RESULTS (see also Tables 3.6 and 3.7)

	P1	P2	P3	P4	P5	alone
N1	7.5	11.5	16.5	7	6.8	13
N2	6.2	8.5	7.8	7.5	13.9	9.3
N3	9.4	6.6	10.9	11.7	9.8	5.9
N4	9.5	7	8.3	7.8	14.4	8.3
N5	6.3	14.3	6.7	6.2	6.9	8
alone	14.7	13.6	6.1	7.3	6.1	

Table 3.6.

	P6	P7	P8	P9	P10	P11	P12	P13	alone
N6	12.1	14.2	22.5	3.5	17	10.6	7.5	5.7	5.1
N7	7.7	8.5	8.4	9.3	16	11.2	4.3	4.5	4.2
N8	7.6	9	3.1	10.5	9.2	7.3	10.8	6.4	3.1
N9	5.7	5.3	5.6	5.4	13.8	7.3	8.8	5	7.7
N10	10.6	5.2	25.5	8.5	14.4	12	10.5	18	3
alone	6.4	11.5	14.9	12.7	27	24	19	14.1	

Table 3.7.

	P14	P15	P16	P17	alone
N11	11.6	8.8	8.6	10.2	10.6
N12	14.3	6.8	5.2	7.2	8.4
N13	6.2	8.4	7	8.9	12.2
N14	14.2	6.1	5	3.6	19.2
alone	12	19	13	15	

N.B. The positive bindings are underlined

Key: P = pericarditis

N = normal

Patient Studies

Patient S.G. Case 1

This 45 year old female had had recurrent attacks of erythema multiforme on her forearm, hands, feet and buccal mucosa since 1977. Each attack was usually preceded by herpes simplex on her lips. Since her illness started she was never free from the erythematous rash. She was treated with intramuscular injections of ACTH. The patient was infused on two occasions (15.5.80 and 29. 7.80) with plasma from her son (S.G.) from one unit each time.

Investigations:

Hb 14g/100ml, WBC 6.5 x 10^9/l, blood virology negative. Other immunological investigations are shown in Table 3.8.

Table 3.8 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT S.G.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
15. 5.80 1 hour Pre T1	100	ND	ND	1.09	3.74	±
2. 6.80 17 days Post T1	96	98	ND	0.4	5.48	-
3. 7.80 48 days Post T1	93	76	ND	0.54	6.90	-
16.10.80 1 day Pre Ig inj.1	88	86	22.3	0.2	2.9	-
30.10.80 13 days post Ig inj.1	82	70	13.2	0.16	2.76	-

N.B. - Abnormal values of C3 and CH50 in all tables are underlined
- Complexed C1q, IgG and IgM in all tables are underlined
- ND = Not Done
- T = Transfusion

Cross-reaction results: The donors: (1) S.G.(son), (2) J.G.(daughter).

1. The result of cross-reaction between the patient and her son by RIA was negative.
2. The cross-reactions between the patient and the donors by the delayed technique are shown in Table 3.9.

Table 3.9 CROSS-REACTIONS BY DELAYED TECHNIQUE

S.G.	S.G.(son)	J.G.	
0.98	2.6	0.43	alone
S.G.	3.7	2.1	mixture
	1.3	0.97	expected

N.B. - Positive reactions in all tables are underlined

Results of treatment:

The first plasma infusion (15.5.80) relieved her erythematous rash and herpes simplex completely, and she remained in complete remission for 2 months. She lost her IgG complexes after infusion. IgM in circulating complexes appeared by 48 days. The patient responded to the second infusion (29.7.80) and remained in complete remission for 2 months after which she relapsed. Intramuscular injection of immunoglobulin (500 mg) was started (17.10.80) for the treatment of the second relapse. This induced complete remission for 11 weeks. The third relapse was treated with 250 mg of immunoglobulin but this did not induce complete remission as a few spots persisted.

Patient T.H. Case 2

This 36 year old male had had recurrent attacks of erythema multiforme on his hands, knees and dorsum of the feet since 1977. He continued to have an acute attack of erythematous rashes once monthly, this was usually preceded by herpes simplex 2 weeks prior to the appearance of erythematous lesion. He was treated with high doses of steroids. Blood virology was negative, titre against herpes hominis was 1/40. Other immunological investigations are shown in Table 3.10. The patient was infused with 1 unit of plasma from his father F.H. on 10.1.80.

Table 3.10 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT T.H.		% normal		PEG Precipitable (%)			
date		C3	CH50	C1q	IgG	IgM	IgA
20. 9.79	110 days Pre T	98	<u>72</u>	6.8	0.35	0.6	-
25.10.79	75 days Pre T	100	<u>64</u>	0	0.1	0	-
2.11.79	68 days Pre T	98	104	ND	0.62	<u>7.6</u>	<u>+</u>
10. 1.80	30' Pre T	78	130	29.9	0.31	3.9	-
4. 3.80	54 days Post T	98	<u>70</u>	36.7	0.3	3.9	-
17. 3.80	67 days Post T	114	104	0	0.26	0	-

<u>Cross-reaction results:</u> The donor: F.H. (father).

1. The cross-reaction between the patient and the donor by RIA is shown in Table 3.11.

Table 3.11 CROSS-REACTION BY RIA

	F.H.	normal
T.H.	<u>0.55</u>	-ve

N.B. - T.H. is the labelled sample
- Positive reactions in all tables are underlined

2. The cross-reaction between the patient and the donor by the delayed technique is shown in Table 3.12.

Table 3.12 CROSS-REACTION BY DELAYED TECHNIQUE

T.H.	F.H.	Mixture	expected
1.8	1.3	1	1.7

<u>Results of treatment:</u>

Erythematous rash and herpes simplex were completely cleared 1 week after plasma infusion until August, 1980, then he had developed a very mild attack of erythematous rash. This was treated with a single intramuscular injection of immunoglobulin after which he felt better and the rash disappeared. He had developed a new herpes lesion in November 1980, which was treated with another injection of immunoglobulin, and this induced remission with no recurrence over the next 3 months.

Patient M.O. Case 3

This 40 year old female had had recurrent attacks of erythema multiforme on her hands and feet since 1978. These were usually preceded by herpes simplex ten days prior to the appearance of the erythematous lesion. She continued to have about 4 attacks of erythematous rashes a year. She was infused with 1 unit of plasma from her son (P.O.) on 24.4.80.

Blood virology was negative and titre against herpes hominis was 1/40. No virus particles could be seen on skin biopsy by electron microscopy. Other blood investigations were normal. Other immunological investigations are shown in Table 3.13.

Table 3.13 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT M.O.		% normal		PEG Precipitable (%)			
date		C3	CH50	C1q	IgG	IgM	IgA
10. 7.79	284 days Pre T	107	91	35.5	0.5	6.7	-
4. 1.80	110 days Pre T	112	106	11	2.27	0	-
1. 2.80	83 days Pre T	110	126	16.5	0.21	3.53	-
3. 5.80	9 days Post T	190	85	ND	ND	3.98	-

Cross-reaction results: The donor: P.O. (son).

1. The cross-reaction between the patient and the donor by RIA was negative.
2. The cross-reaction between the patient and the donor by the delayed technique is shown in Table 3.14.

Table 3.14 CROSS-REACTION BY DELAYED TECHNIQUE

M.O.	P.O.	Mixture	expected
0.82	1.18	3.6	0.89

Results of treatment:

Erythematous rash and herpes simplex were completely cleared a few days after plasma infusion and the patient remained in complete remission until February 1981.

Patient J.S. Case 4

This 25 year old male had had recurrent attacks of erythema multiforme on his hands and feet since 1973. He continued to have acute attacks of erythematous rashes three times a year. He was treated with prednisolone and intramuscular injections of ACTH. The patient was infused with 1 pint of fresh frozen plasma on 7.3.80.

Investigations:

WBC 7.9 x 10^9/l, RBC 5.3 x 10^{12}/l, ESR 2, titre against herpes hominis was 1/40. Other blood virology was negative. Other immunological investigations are shown in Table 3.15.

Table 3.15 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT J.S.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
6. 3.80 1 day Pre T	98	96	55.4	0.38	4.26	-
12. 3.80 5 days Post T	120	114	67.2	0.55	11.3	-
20. 3.80 13 days Post T	117	100	16.8	0.33	3	-
11. 6.80 94 days Post T	92	90	ND	0.28	1.37	-
25. 6.80 108 days Post T	102	80	ND	0.28	3.13	-

Cross-reaction results:

The cross-reaction between the patient and the fresh frozen plasma by the delayed technique is shown in Table 3.16.

Table 3.16 CROSS-REACTION BY DELAYED TECHNIQUE

J.S.	FFP	Mixture	expected
1.77	2.16	3.15	1.84

Results of treatment:

There was no clinical benefit from the infusion. IgM complexes were found by 5 days post infusion and then disappeared. C1q in circulating complexes disappeared by 13 days.

Patient F.A. Case 5

This 24 year old female with lupus nephritis had had polyarthritis, rashes over the face and the arms and hypertension since 1975. Azathioprine and prednisolone did not control her symptoms. The patient was treated with plasmapheresis on (1.10.75).

Investigations:

Plasma creatinine 167 μmol/l, ANF positive, DNA binding 33 units/ml, 24 hour urinary protein 12 g. Other immunological investigations are shown in Table 3.17.

Results of plasmapheresis:

Six plasmaphereses with plasma protein fraction (PPF) were performed. Rash and joint pain subsided, 24 hour proteinuria fell from 13 to 8 g following treatment. IgG in circulating complexes fell after exchange but remained above normal, C1q in circulating complexes fell after some exchanges, C3 and CH50 levels remained low (Fig. 3.8). She relapsed after 8 weeks and 3 exchanges in 8 weeks with fresh frozen plasma (FFP) were performed. C3 levels rose to normal. C1q and IgG in circulating complexes fell after plasma exchange. Proteinuria fell to 2.5 g in 24 hours. She relapsed after 10 weeks and was treated with further exchanges with fresh frozen plasma after which the circulating complexes were not found (Fig. 3.8). Two months later she died in hypertensive heart failure.

Table 3.17 COMPLEMENT AND IC DATA BEFORE AND AFTER PLASMAPHERESIS

PATIENT F.A.	% normal		PEG Precipitable (%)	
date	C3	CH50	C1q	IgG
1.10.75 Pre PP1	39	12.2	42.5	2.5
1.10.75 Post PP1	19	ND	37.8	1.5
6.10.75 Pre PP2	29	9.4	62.4	1.8
6.10.75 Post PP2	18	5.5	29	0.8
9.10.75 Pre PP3	32	7.5	29.75	1.7
9.10.75 Post PP3	16	10	37.8	1.7
(No immediate pre PP4 sample available)				
15.10.75 Post PP4	47	6.7	40	0.85
22.10.75 Pre PP5	74	14.6	79	1.7
22.10.75 Post PP5	42	0	90.75	0.85
24.10.75 Pre PP6	63	19.2	90.75	1.86
24.10.75 Post PP6	42	13.1	76.1	1.15
10.12.75 Pre PP7	ND	ND	90	1.31
10.12.75 Post PP7	ND	ND	56.4	0.58
9. 2.76 Pre PP8	77	30	91.25	1.83
9. 2.76 Post PP8	ND	ND	77.4	1.15
10. 2.76 Post PP8	112	ND	61.8	0.99
10. 2.76 Post PP8	95	20	82.85	1.33
12. 2.76 Pre PP9	89	47	27.8	0.43
12. 2.76 Post PP9	86	82	14.4	0.65
13. 2.76 Post PP9	79	36	43.8	0.5
13. 2.76 Post PP9	84	25	27.8	0.55
15. 2.76 Post PP9	76	18	34.4	0.35
16. 2.76 Post PP9	79	18	34.4	0.5
17. 2.76	84	23	41.35	1
15. 3.76	ND	15	23.35	0.7
23. 3.76	59	39	65.85	1.61
3. 4.76	62	21	68.75	1.32
8. 4.76	54	14	66.7	1.32
10. 4.76	48	29	69.5	2.75
11. 4.76 Pre PP10	47	18	0	0.38
12. 4.76 Post PP10	46	20	39	1.53
13. 4.76 Post PP10	79	35	34.5	1.03
14. 4.76 Post PP10	92	81	61.5	0.7

/ contd...

Table 3.17 COMPLEMENT AND IC DATA BEFORE AND AFTER PLASMAPHERESIS (Contd.)

date	% normal		PEG Precipitable (%)	
	C3	CH50	C1q	IgG
17. 4.76	47	34	59	1.08
18. 4.76	53	31	19.5	2.5
19. 4.76 Pre PP11	64	32	21.5	0.55
19. 4.76 Post PP11	58	51	21.5	0.45
22. 4.76	51	19	15.5	0.45
23. 4.76 Pre PP12	58	17	36.5	0.54
23. 4.76 Post PP12	47	15	14	0.25
24. 4.76 Pre PP13	58	43	31	0.39
24. 4.76 Post PP13	50	24	19	0.25
26. 4.76 Pre PP14	61	18	40	0.55
26. 4.76 Post PP14	49	13	12.5	0.20
27. 4.76 Pre PP15	50	15	23.5	0.30
27. 4.76 Post PP15	45	13	14	0.25
28. 4.76	48	37	0	0.23
29. 4.76	45	34	10.4	0.21
30. 4.76	47	31	50.3	0.23
3. 5.76	58	39	61	0.46
6. 5.76	61	42	63.5	0.39
11. 5.76	73	59	55.5	0.46

N.B. - PP = Plasmapheresis

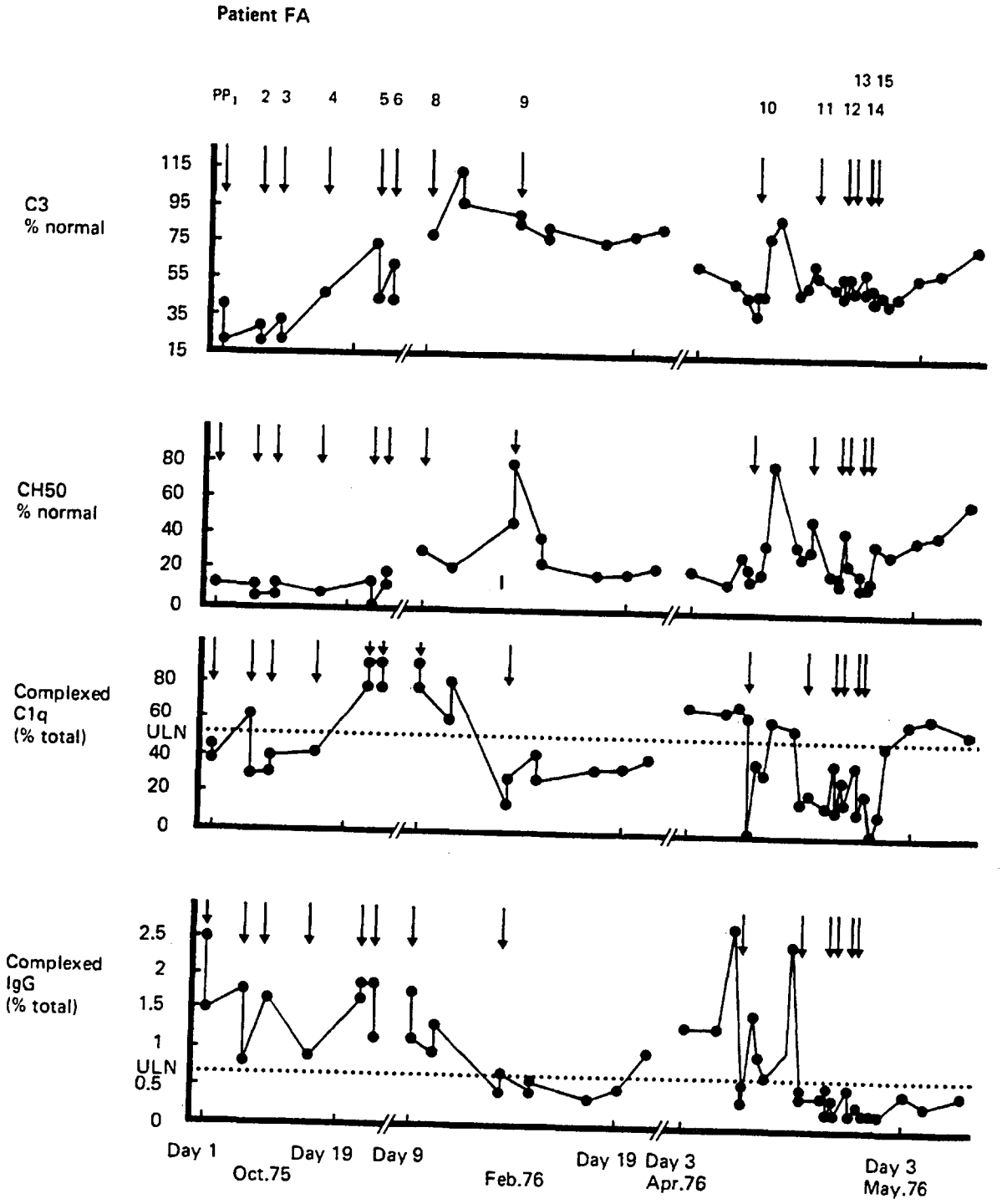


Fig 3.8
Complement and circulating complexes before and after treatment

Patient F.M. Case 6

This 31 year old female from the Philippines was admitted to U.C.H. on 15.10.77 with a ten day history of oliguria and generalized oedema. The oedema had started after an upper respiratory tract infection. The patient had a renal biopsy performed which showed membranoproliferative glomerulonephritis (mesangiocapillary glomerulonephritis). She was treated with diuretics and had received two units of blood. She was discharged on 30.11.77. Prednisolone was started in November 1978. The patient was readmitted on 11.12.78 because of uncontrolled nephrotic syndrome and SLE was diagnosed. She was infused on two occasions (19.1.79 and 30.4.79) with plasma from her brother (M.M.).

On examination:

CNS and CVS normal. Chest clear. BP 140/95 mm Hg. No rashes. Oedema of arms, legs, abdomen and face.

Investigations:

WBC 3900/mm^3, Hb 12.3 g/100 ml, ESR 57, platelets 146,000, urea 20.7 mmol/l, plasma creatinine 140 µmol/l, total plasma protein 38 g/l, albumin 21 g/l, globulin 17 g/l, creatinine clearance 44 ml/minute, ASO less than 200 units, ANF was negative but later became positive, Australia antigen negative, MSU sterile but contained moderate numbers of RBC, 24 hour urine protein excretion 5 g, DNA binding became 40 units/ml by the end of 1978. Other immunological investigations are shown in Table 3.18.

Table 3.18 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION:

PATIENT F.M.		% normal		PEG Precipitable (%)			
date		C3	CH50	C1q	IgG	IgM	IgA
25.10.77	15 days Pre BT	46	28	60	1.2	ND	-
31.10.77	10 days Pre BT	29	0	21.5	1.25	ND	ND
8.11.77	2 days Pre BT	26	0	8.5	0.65	ND	ND
24.11.77	14 days Post BT	31	0	21.5	1.07	ND	ND
28.11.77	18 days Post BT	34	0	35	0.95	ND	ND
16.12.77	36 days Post BT	38	0	39	0.82	ND	ND
30. 1.78	50 days Post BT	87	105	70	0.65	ND	-
27. 2.78	77 days Post BT	85	85	65	0.45	ND	-
30. 3.78	120 days Post BT	88	99	70	1.14	ND	+
10. 4.78	130 days Post BT	96	88	95	1.3	4	-
12. 6.78	192 days Post BT	90	95	77.5	2.2	40	-
11.10.78	98 days Pre PT1	91	60	33	0.4	0	-
12.11.78	67 days Pre PT1	73	20	115	1.2	13	+
13.12.78	36 days Pre PT1	67	30	17	0.3	1.7	+
14.12.78	35 days Pre PT1	56	30	34	0.2	2.1	+
26. 1.79	7 days Post PT1	74	100	22	0.5	1.3	+
9. 4.79	21 days Pre PT2	86	90	50.7	1.2	7	±
10. 4.79	20 days Pre PT2	89	102	62.9	1.1	5.3	-
11. 4.79	19 days Pre PT2	85	60	54	0.95	5.8	+
30. 4.79	30' Pre PT2	102	40	44	0.87	5	-
30. 4.79	30' Post PT2	63	40	10.2	0.77	2.9	-
3. 5.79	3 days Post PT2	66	30	34	0.8	3.6	±
9. 5.79	9 days Post PT2	75	30	34	1	3.9	±
18. 5.79	18 days Post PT2	67	30	63	0.72	3.4	-
21. 5.79	21 days Post PT2	70	56	47	0.44	2.6	±
29. 5.79	29 days Post PT2	82	65	68	0.81	5.7	-
12. 6.79	42 days Post PT2	81	96	47	0.2	3.8	-
14. 6.79	44 days Post PT2	70	75	58	0.5	1.9	±
25. 6.79	55 days Post PT2	80	120	71.5	0.47	3.8	-

N.B. - BT = Blood Transfusion

- PT = Plasma Transfusion

Cross-reaction results: The donors: (1) M.M.(brother), (2) A.M.(brother).

a. The cross-reactions between the patient and the donors by RIA are shown in Table 3.19.

Table 3.19 CROSS-REACTIONS BY RIA:

	F.M.	M.M.	A.M.
F.M.	6.8	1.9	-ve
M.M.	4.2	1.1	1.5
A.M.	2.9	-ve	-ve
J.B.K. (normal)	0.7	-ve	-ve
A.K. (normal)	-ve	ND	ND

N.B. - The horizontal rows related to each sample used to coat a cup, and the vertical columns related to each labelled complex added to the cups.

b. The cross-reactions between the patient and the donors by the delayed technique were insignificant because the donors' samples were old and IgG was not found in their PEG pellet.

Results of treatment:

On admission the patient's renal function was poor. Generalized oedema was present. Urinary output was low. She was treated with diuretics and infused with 2 pints of stored blood (10.11.77), after which she had developed diuresis, oedema was gradually resolved and renal function began to improve. The patient remained in complete remission until November 1978 after which she relapsed and her kidney function started to deteriorate. She was infused with 2 pints of plasma (19.1.79); this induced complete remission, her kidney function started to improve, for example, her creatinine clearance rose from 12 ml/min before transfusion up to 22 ml/min by fifth day post-transfusion, and a ten week remission was obtained. Immune complex levels remained undetectable, CH50 and C3 levels returned to normal (Fig.3.9). The second relapse was treated with 1 pint of plasma (30.4.79) but she did not respond. Four months later she died of septicaemia.

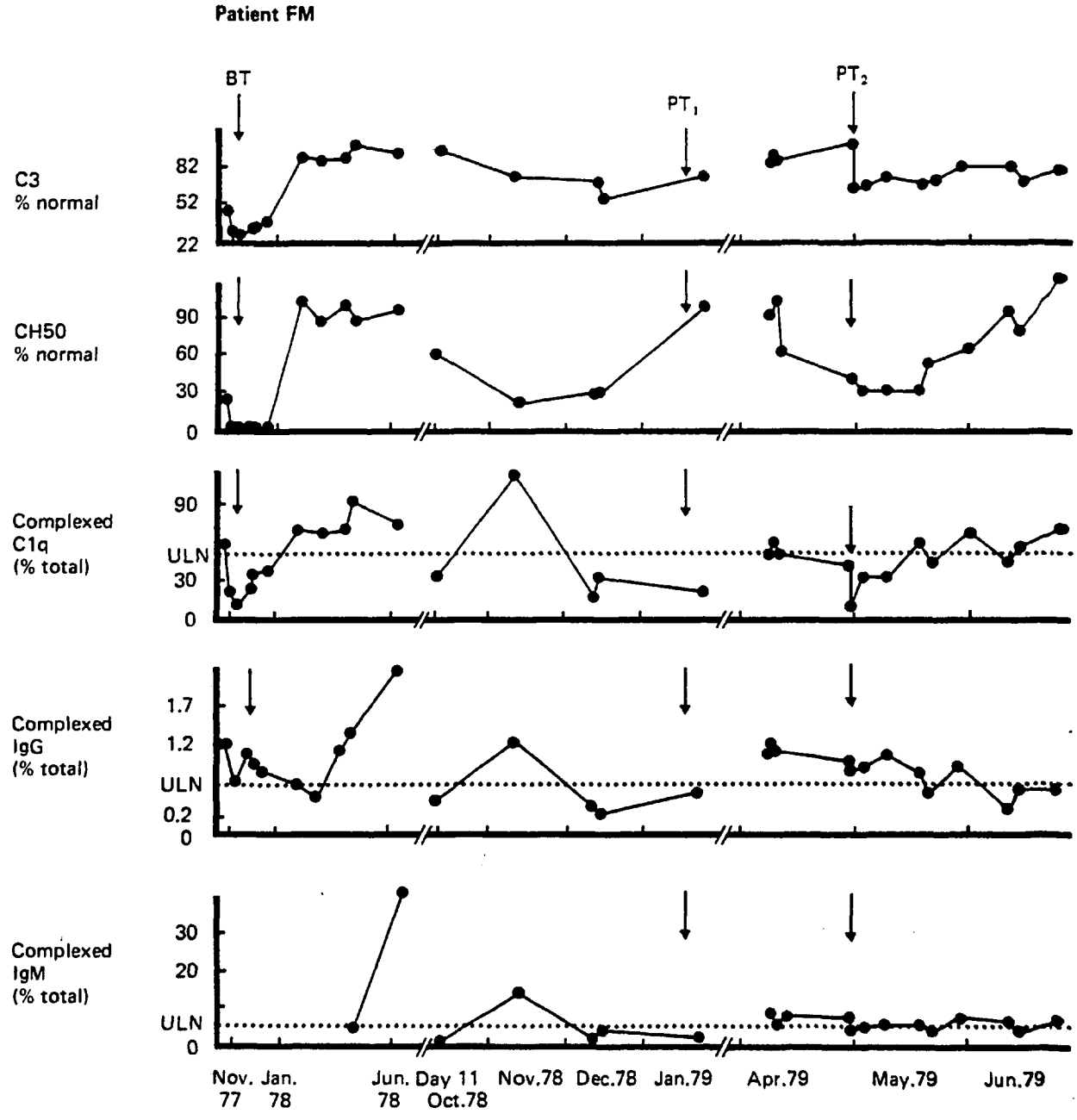


Fig 3.9
Complement and circulating complexes before and after treatment

Patient W.M. Case 7

This 55 year old lady with systemic lupus erythematosus had had polyarthritis, malaise and facial rashes since 1965. Prednisolone and anti-inflammatory agents did not control her joint symptoms.

The patient was treated with plasmapheresis in 1976.

Investigations:

ANF +ve, DNA binding 32 units/ml, LE cells positive. Other immunological investigations are shown in Table 3.20.

Results of plasmapheresis:

Five exchanges with plasma protein fraction reduced her pain but morning stiffness did not improve. Complement and immune complex levels fell after exchange. Four weeks later four plasmaphereses were performed with fresh frozen plasma (FFP). After the treatment morning stiffness subsided, CH50 levels rose, and C1q and IgG complex levels fell (Fig.3.10). Malaise returned after one month and she relapsed after three months.

Table 3.20 COMPLEMENT AND IC DATA BEFORE AND AFTER PLASMAPHERESIS

PATIENT W.M.		% normal		PEG Precipitable (%)	
date		C3	CH50	C1q	IgG
8.12.75	Pre PP1	90	39	53.6	0.5
14. 1.76	Pre PP1	79	132	68	0.65
(no immediate Post PP1 sample available)					
28. 1.76	Pre PP2	113	168	89.5	0.51
28. 1.76	Post PP2	67	63	55.4	0.37
4. 2.76	Pre PP3	51	ND	30.8	0.24
(no immediate Post PP3 sample available)					
11. 2.76		35	33	39	0.75
13. 2.76		93	71	64.4	0.55
18. 2.76	Pre PP4	89	48	51.2	0.65
18. 2.76	Post PP4	64	56	43.75	0.35
25. 2.76		76	71	64.45	0.35
3. 3.76	Pre PP5	93	72	87.45	1.1
3. 3.76	Post PP5	73	21	58.95	0.55
10. 3.76		83	80	78	0.6
17. 3.76		87	80	80.7	0.53
6. 4.76	Pre PP6	94	70	100	0.9
6. 4.76	Post PP6	92	71	65.85	0.70
7. 4.76	Pre PP7	103	57	71.7	0.70
7. 4.76	Post PP7	87	74	23	0.57
8. 4.76		87	92	36.5	0.75
9. 4.76	Pre PP8	74	75	59.2	0.73
(no immediate Post PP8 sample available)					
11. 4.76		92	90	78.5	1.3
12. 4.76		79	102	57	0.8
14. 4.76		85	116	78.5	1.2
5. 5.76		93	76	123.5	1.3
1. 7.76		77	39	65	1.1
23.11.76		102	26	46	0.32
24.11.76		94	20	3	0.22

N.B. - No date available for the 9th plasmapheresis.

Two patients F.A. and W.M. who had been studied previously in this department were briefly described by Moran, Parry, Mowbray, Richards and Goldstone (1977).

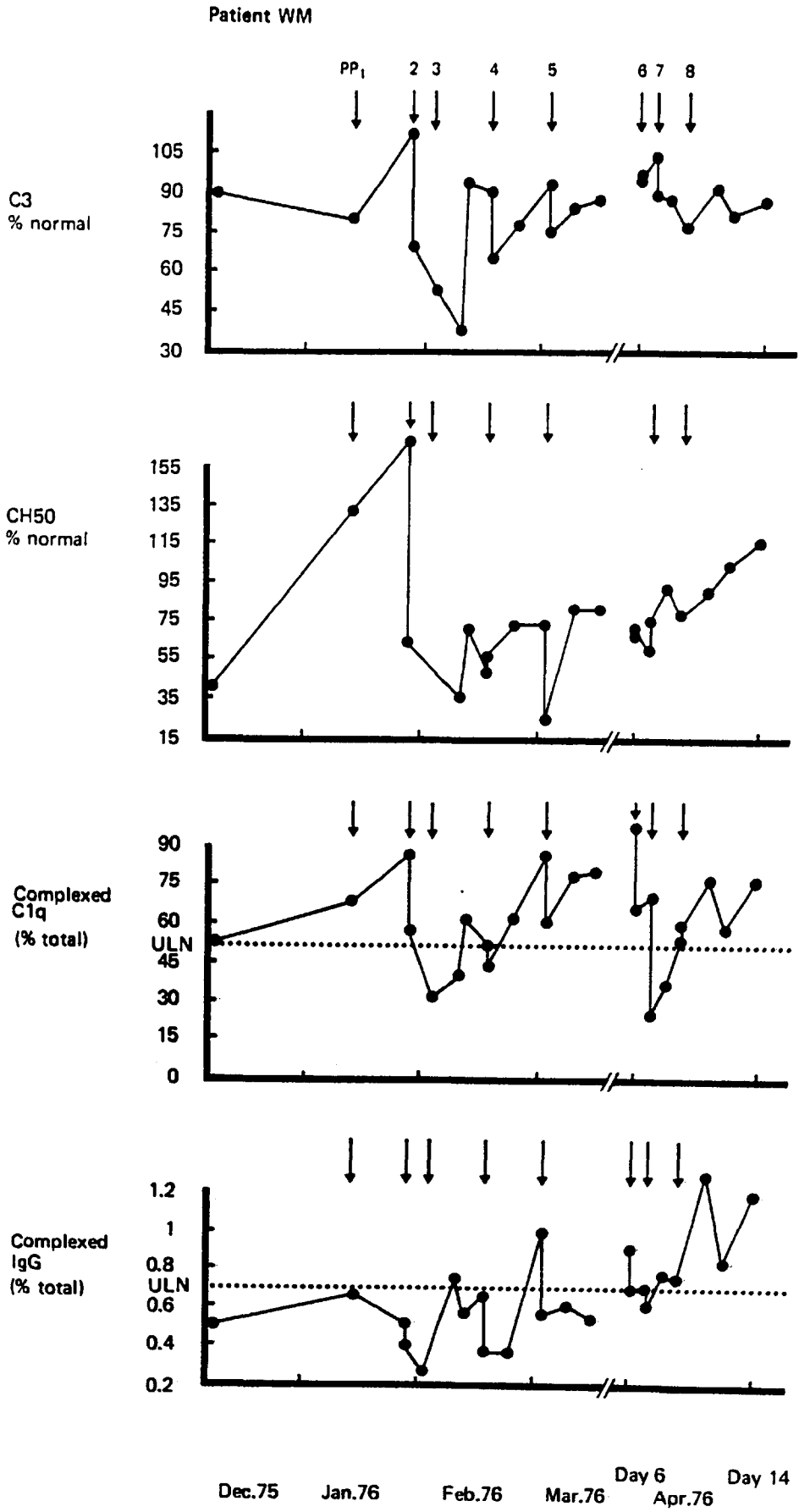


Fig 3.10
Complement and circulating complexes before and after treatment

Patient F.B. Case 8

This 32 year old male with congenital deafness was admitted to U.C.H. on 2.5.75 with a 2 month history of lethargy, arthropathy in the large joints and purpuric rash over his ankles. He was found to have proteinuria and haematuria as well. Renal biopsy and skin biopsy were performed. Wegener's granulomatosis was diagnosed, for which he had been given prednisolone and dapsone. The patient was infused with one pint of plasma from his wife (P.B.) on 12.3.1980.

On examination:

He was deaf, tired and slightly pale. He had nasal obstruction. There was no oedema. BP 120/80 mm Hg. CNS and CVS normal.

Investigations:

WBC 8.5 x 10^9/l, RBC 3.29 x 10^{12}/l, Hb 10.2 g/100 ml, ESR 60, plasma protein 8.5 g/100 ml, albumin 3.7 g/100 ml, globulin 4.8 g/100 ml, IgG 2200 mg/100 ml, IgM 108 mg/100 ml, IgA 290 mg/100 ml, liver function tests normal, blood urea normal, plasma creatinine normal, ANF negative, rheumatoid factor positive, Australia antigen negative in the serum but positive in the skin biopsy, MSU sterile but contained few red blood corpuscles, 24 hour urine protein excretion 0.1 g, renal biopsy showed focal glomerulonephritis. Other immunological investigations are shown in Table 3.21.

Table 3.21 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT F.B.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
6. 6.75 4y, 9m Pre T	94.5	42.9	40	0.54	ND	ND
17. 7.75 4y, 8m Pre T	116	112	28.5	0.46	ND	ND
8. 8.75 4y, 7m Pre T	106	71	27	0.36	ND	ND
15.10.75 4y, 5m Pre T	110	0	17	0.38	ND	ND
14. 1.76 4y, 2m Pre T	95	55	103.2	1	ND	ND
19. 1.76 4y, 2m Pre T	128	132	47.85	0.99	ND	ND
15. 3.76 4y Pre T	83	57	63.75	0.58	ND	ND
14. 6.76 3y, 9m Pre T	91	100	69	0.85	ND	ND
13.12.76 3y, 3m Pre T	83	69	41	0.89	ND	ND
12.12.77 1y, 3m Pre T	80	99	58.5	0.65	ND	ND
6. 3.78 2y Pre T	89	91	44.5	0.45	ND	ND
2. 8.78 1 y, 7m Pre T	97	90	28.7	0.7	3.3	-
3. 3.80 9 days Pre T	106	140	79.3	0.83	4.66	-
12. 3.80 30' Post T	115	102	25.9	0.88	5.66	ND
13. 3.80 1 day Post T	114	110	24.5	0.66	5.09	ND
19. 3.80 7 days Post T	119	114	13.2	0.44	2.7	ND
26. 3.80 15 days Post T	117	90	18	0.87	3	±
16. 4.80 1m, 4 days Post T	106	100	31	0.4	0.53	±
12. 5.80 2m Post T	100	76	ND	0.48	4.2	-

N.B. - y = year; m = month

Cross-reaction results: The donor: P.B. (wife)

a. The cross-reaction between the patient and the donor by RIA is shown in Table 3.22.

Table 3.22 CROSS-REACTION BY RIA:

	F.B.	P.B.	A.K. (normal)
F.B.	-ve	0.62	-ve

N.B. - F.B. in horizontal row is the labelled sample

b. The cross-reaction between the patient and the donor bythe delayed technique is shown in Table 3.23.

Table 3.23 CROSS-REACTION BY DELAYED TECHNIQUE:

F.B.	P.B.	mixture	expected
2.5	1.64	3	2.3

Results of treatment:

Plasma infusion cleared his rash for 5 days after which he had developed new spots. It seems that there was no clinical benefit from plasma infusion. He lost his circulating complexes after transfusion, but his IgG complexes appeared again by 15 days (Fig. 3.11).

Patient I.J. Case 9

This 53 year old lady had had generalized weakness, malaise, polyarthritis, Raynaud's syndrome and facial and trunk rashes since 1976. Mixed connective tissue disease was diagnosed, for which she was treated with prednisolone and anti-inflammatory agents. She was infused with 2 pints of fresh frozen plasma on 5.3.1979.

On examination:

All systems were normal. BP was 110/80.

Investigations:

RBC 3.9 x 10^{12}/1, WBC 5.9 x 10^{9}/1, Hb 12.4 g/100 ml, ESR 63, liver function tests and kidney function tests normal, ANF negative, DNA binding 10 units/ml, antibodies to ENA positive. Other immunological investigations are shown in Table 3.24.

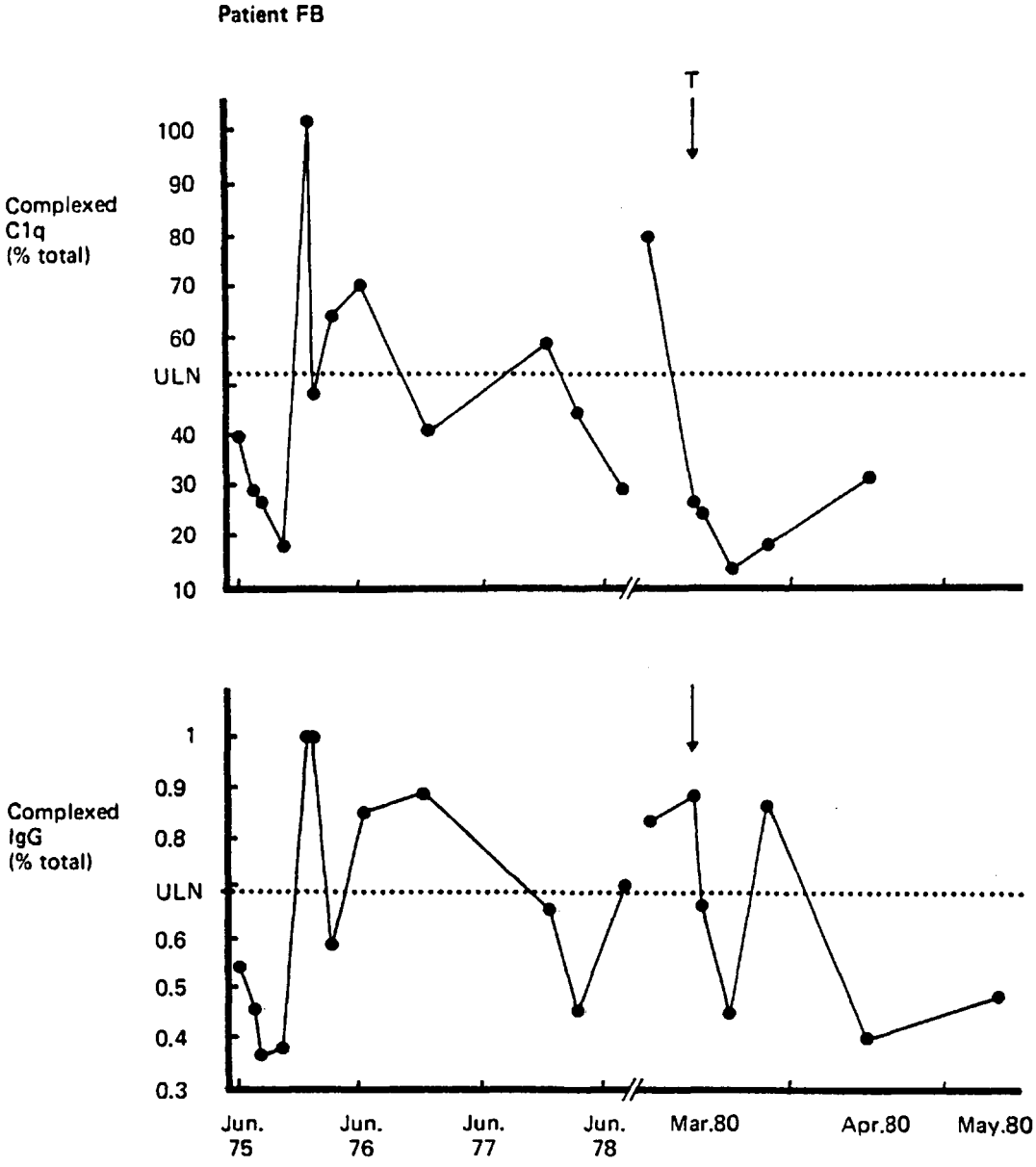


Fig 3.11
Circulating complexes before and after treatment

Table 3.24 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT I.J.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
26. 6.77 1y, 9m Pre T	108	90	65	0.82	ND	±
15. 7.77 1y, 8m Pre T	102	110	13	0.65	ND	-
19. 6.78 8m, 16 days Pre T	82	65	97	1.45	7.4	-
15.11.78 3m, 20 days Pre T	134	50	62	1.86	4.2	-
9. 3.79 4 days Post T	95	112	15	0.46	1.7	-

Results of treatment:

The patient was admitted to St. Mary's Hospital for the acute attack of her illness; she was treated with 2 pints of fresh frozen plasma. A striking improvement occurred after the treatment. Malaise and joint pain disappeared and she was in complete remission when discharged a few weeks later. Arthritis and malaise returned after 10 months. 4 Days post infusion, circulating immune complexes were not found.

Patient R.J. Case 10

This 41 year old male was admitted to U.C.H. in 1977 for further investigations of proteinuria and fatigue. Since 1968 he had suffered from arthritis of his interphalangeal, metacarpophalangeal, wrist, elbow, shoulder, hip and knee joints. Rheumatoid arthritis was diagnosed, for which he had been given a variety of drugs including steroids and gold injections. He had had bilateral total hip replacements in 1973. He was lost to hospital follow-up until 1977. Renal biopsy was performed in 1979. The patient was infused with one pint of plasma from his brother (W.J.) on 21.8.79.

On examination:

CVS and CNS normal. There were crepitations at the lung bases. BP 140/95 mm Hg. Metacarpophalangeal and proximal interphalangeal

joints were swollen. Elbow, shoulder and knee joints were painful. Oedema was not present.

Investigations:

WBC 6300/mm^3 RBC 3.4 x 10^{12}/l, ESR 24, Hb 10.3 g/100 ml, serum creatine 136 µmol/l, serum urea 16.9 mmol/l, creatinine clearance 30 ml/min, cholesterol 5.6 mmol/l, DNA binding 5 U/ml, Rose-Waaler and antinuclear factor positive, Australia antigen negative, MSU sterile and contained no RBC, 24 hour urine protein excretion 1.16 g; renal biopsy showed amyloidosis and renal vasculitis. Other immunological investigations are shown in Table 3.25.

Table 3.25 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT R.J.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
7. 4.78 1y, 4m Pre T	96	115	19.4	0.49	18	+
17. 7.78 1y, 1m Pre T	104	94	14.8	4.2	25	+
11. 9.78 11m, 10 days Pre T	104	92	20.3	4.1	22	+
28. 2.79 5m, 23 days Pre T	105	98	61.7	2	7.2	±
9. 5.79 3m, 12 days Pre T	102	106	58.5	1.4	7.3	±
30. 5.79 2m, 21 days Pre T	ND	69	5.1	0.23	1.3	±
25. 6.79 1m, 25 days Pre T	107	116	61.2	1.1	9.4	±
9. 7.79 1m, 11 days Pre T	78	80	12.2	1.8	11	-
21. 8.79 30' Pre T	84	108	51.5	1.3	12.5	+
21. 8.79 1 hour Post T	82	86	40.8	0.54	6.3	ND
24. 8.79 3 days Post T	64	86	69	1	7.6	+
29. 8.79 8 days Post T	98	91	69	1.2	8.9	-

Cross-reaction results: The donors, (1) W.J.(brother), (2) P.J.(wife)

1. The results of cross-reactions between the patient and the donors by RIA are shown in Table 3.26.

Table 3.26 CROSS-REACTIONS BY RIA:

	R.J.	P.J.	W.J.
R.J.	0.7	1.2	1.4
P.J.	0.2	-ve	ND
W.J.	0.5	ND	-ve
J.F.M. (normal)	-ve	ND	ND
A.S. (normal	-ve	ND	ND

b. The cross-reaction between the patient and the donor W.J. by the delayed technique is shown in Table 3.27.

Table 3.27 CROSS-REACTION BY THE DELAYED TECHNIQUE:

R.J.	W.J.	mixture	expected
4.16	2.17	3.24	3.76

Results of treatment:

There was no clinical improvement in the patient and his kidney function stayed the same. He lost his IgG complexes immediately after the infusion for 3 days. C1q in circulating complexes appeared after 3 days. IgM in circulating complexes fell immediately after the infusion but remained higher than the normal, and began to increase gradually (Fig. 3.12).

Patient F.L. Case 11

This 47 year old lady was admitted to St. Mary's hospital in 1967 complaining of 7 months duration easy bruising, bleeding 2 days after dental extraction, heavy period for 9 months and red spots on her feet and arms. Idiopathic thrombocytopenic purpura was diagnosed. She had had a splenectomy and was treated with steroids to which azathioprine was added. In 1972 hypothyroidism and pernicious anaemia were diagnosed, which were known to be well controlled by a specific treatment. The

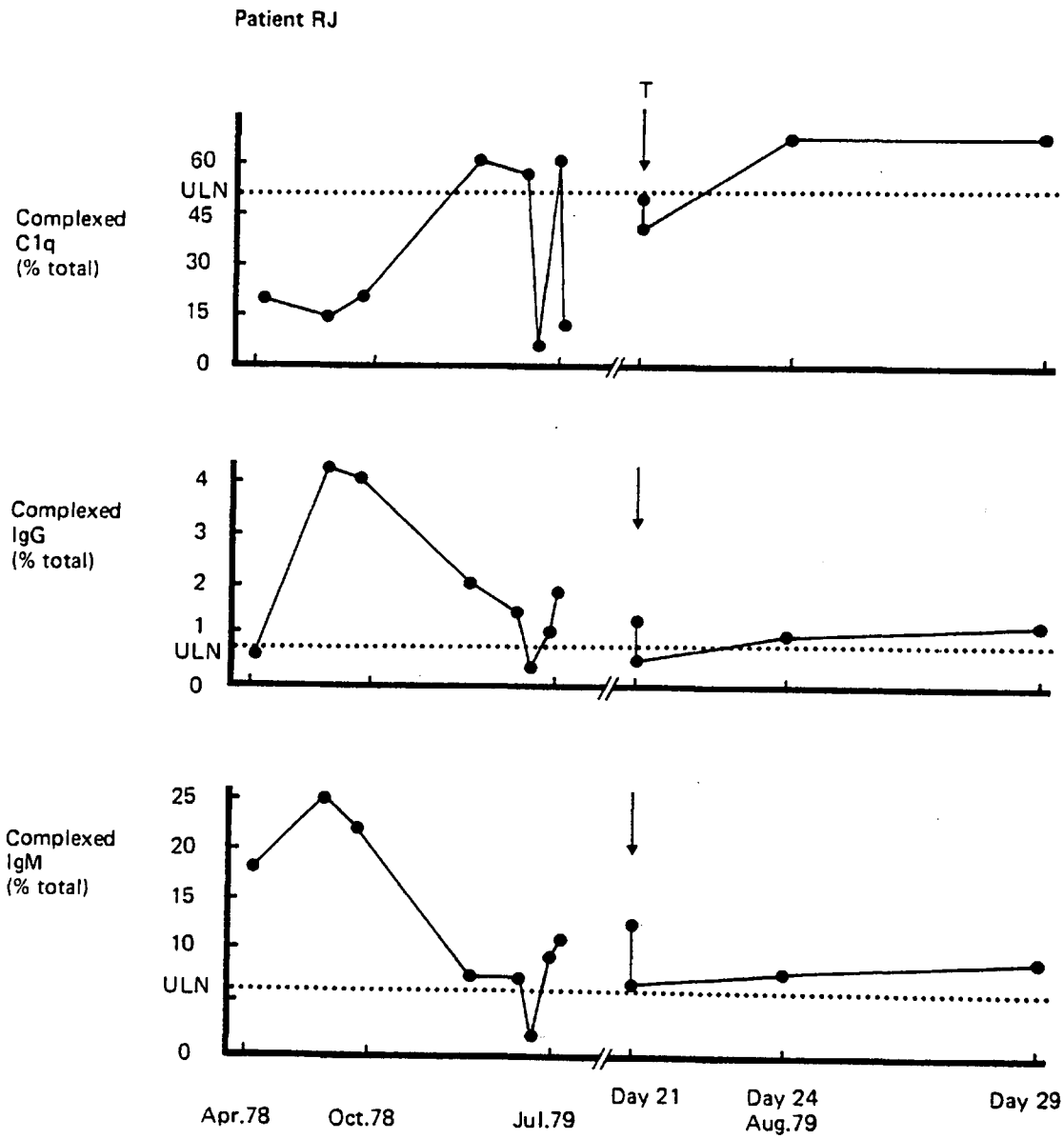


Fig 3.12
Circulating complexes before and after treatment

patient had needed hospitalization from time to time for the idiopathic episodes of her illness which were known to be unreponsive to splenectomy, steroids and immunosuppressive treatments. The patient was infused with 2 units of plasma from her two sons on 8.9.1978.

On examination:

RS, CNS and CVS were normal. BP 130/84 mm Hg. Bruising of the left wrist, purpuric spots on the legs and the abdomen were present.

Investigations:

WBC 6000/mm^3, Hb 10 g/100 ml, ESR 42, PCV 43%, MCHC 30%, kidney function tests and liver function tests normal, prothrombin time 39 seconds with control 14 seconds, platelet counts 135,000, 11,000, 30,000, and 120,000 on different occasions, ANF negative, slide latex negative, DNA binding 11 U/ml. Other immunological investigations are shown in Table 3.28.

Table 3.28 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION:

PATIENT F.L.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
15. 7.78 53 days Pre T	84	97	65	0.98	ND	ND
7. 9.78 1 day Pre T	98	100	105	1.3	4.6	-
8. 9.78 30' Post T	93	86	54	1.22	3.6	+
14. 3.79 186 days Post T	88	104	6.5	0.4	3.6	+

Cross-reaction results: The donors, (1) J.L.(husband, (2) P.L.(son), (3) W.L. (son).

1. The cross-reactions between the patient and the donors by RIA were negative.
2. The cross-reactions between the patient and the donors by the delayed technique were negative and are shown in Table 3.29.

Table 3.29 CROSS-REACTIONS BY DELAYED TECHNIQUE:

F.L.	P.L.	J.L.	W.L.	
2.5	2.05	0.51	1.64	alone
F.L.	1.85	1.54	1.55	mixture
	2.4	2.1	2.2	expected

Results of treatment:

There was no clinical benefit from the treatment and the purpura persisted. Six months later C1q and IgG in circulating complexes were not found.

Patient R.M. Case 12

This 37 year old male had had recurrent attacks of purpura since 1971 which were mainly limited to the lower legs. In 1975 he had experienced a widespread attack of purpura, a biopsy taken at the time was reported as showing features of allergic vasculitis. The eruption responded to a course of systemic steroid; in 1979 he had had a further episode of steroid responsive purpura which recurred when the dose of prednisolone was reduced below 20 mg a day. In 1979 dapsone was added to which he responded but was discontinued after 2 weeks due to falling haemoglobin levels. Another skin biopsy performed in St. John's Hospital for Diseases of the Skin in 1980 suggested dermatitis herpetiformis-like change on the basis of linear IgA deposition but this was not supported by later data. He responded to elimination diet, but not to gluten withdrawal. The patient was infused on three occasions with plasma from his wife on 10.12.79, 6.2.80 and on 29.2.80 from one unit each time.

On examination:

RS, CVS and CNS were normal, inspection revealed eruptions affecting his trunk and limbs. Purpuric macules and papules were located on his

arms and legs, without vesicle formation.

Investigations:

WBC 7700/mm^3, Hb 12.4 g/ml, ESR 40, kidney function tests and liver function test normal, IgG 13 g/l, IgA 2.4 g/l, IgM 1.9 g/l, ANF positive at 1/10, rheumatoid factor positive at 1/80, MSU sterile and negative for RBC and protein. Other immunological investigations are shown in Table 3.30.

Table 3.30 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT R.M.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
5. 9.79 95 days Pre T1	104	40	59.3	0.76	10.8	±
17.10.79 53 days Pre T1	ND	65	90.2	0.94	10.8	±
10.12.70 30' Pre T1	93	90	56	1.26	13.2	±
11.12.79 1 day Post T1	104	72	58.3	1.6	11.4	±
14.12.79 4 days Post T1	101	82	22.3	0.83	10.3	±
6. 2.80 30' Pre T2	112	94	18.1	0.55	3.98	±
7. 2.80 1 day Post T2	116	72	96.7	1.46	15.7	±
11. 2.80 5 days Post T2	115	93	69.3	0.92	7.1	±
18. 8.80 12 days Post T2	101	80	66	0.84	11.8	±
25. 2.80 19 days Post T2	128	92	16.8	0.47	11.9	±
29. 2.80 30' Pre T3	112	75	57.9	0.61	12.7	±
1. 3.80 2 days Post T3	112	62	63.1	0.5	12.12	±
7. 3.80 8 days Post T3	113	76	63.1	0.57	4.9	±
13. 3.80 14 days Post T3	109	88	56	0.88	12	±
25. 3.80 26 days Post T3	123	92	54.2	0.83	12.5	±
31. 3.80 32 days Post T3	102	96	19.8	0.56	20.2	±
8. 4.80 39 days Post T3	99	90	59.8	1.13	17.82	±
28. 4.80 59 days Post T3	97	100	ND	1.31	7.68	±
6. 5.80 67 days Post T3	125	72	ND	1.25	9.7	±

Cross-reaction results: The donors: J.M. (wife), RN, DM, IP, DHM, MP, MO, RE, CP, KC and BG (all friends).

1. The cross-reactions between the patient and the donors by RIA are shown in Table 3.31.

Table 3.31 CROSS-REACTIONS BY RIA:

	J.M.	R.N.	I.P.	DHM	M.P.	M.O.	R.E.	C.P.	K.C.	B.G.	JEM (normal)
RM	0.7	0.71	0.16	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

N.B. - RM is the labelled sample

2. The cross-reactions between the patient and the donors by the delayed technique are shown in Table 3.32.

Table 3.32 CROSS-REACTIONS BY DELAYED TECHNIQUE:

R.M.	J.M.	D.M.	I.P.	DHM	M.P.	R.N.	M.O.	R.E.	
2.63	1.77	1.94	1.94	1.77	1.37	1.33	3.63	1.94	alone
R.M.	4.3	1.7	1.77	1.73	1.94	1.77	1.94	2.16	mixture
	2.5	2.49	2.49	2.45	2.398	2.4	2.8	2.49	expected

Result of treatment:

There was no clinical benefit from the infusions.

Patient F.P. Case 13

This 59 year old lady was admitted to U.C.H. on 23.4.1976 with a history of left leg stiffness and bruising with bright red painful blotches on the anterior aspect 18 months prior to admission. She had a further attack on the right leg, then on both legs. The attacks of erythema subsequently recurred about every 4 weeks and later increased to a 2 week interval. The patient had a skin biopsy performed which showed cutaneous vasculitis of unknown origin. She was on prednisolone (15 mg) since 1976 to which dapsone was added in 1979. She was followed up in the out-patients' clinic. The patient was infused on 3 occasions with plasma from 1 unit each time. The first infusion was on 30.5.1979 from her brother (A.P.), the second and third infusions were on 18.6.1979 and 21.3.80 from her sister (L.G.).

On examination:

Round, deep red discoloration over the dorsum of both feet could be seen and the skin was acutely tender and surrounded by erythema. RS, CVS and CNS normal. BP 130/80 mm Hg.

Investigations:

WBC 3.3 x 10^9/l, RBC 3.4 x 10^{12}/l, Hb 11.7 g/100 ml, ESR 24. Kidney function tests and liver function tests normal. ANF negative, rheumatoid factor negative, Australia antigen negative, plasma protein 74 g/l, albumin 40 g/l, globulin 34 g/l, IgG 11 g/l, IgA 2.3 g/l, IgM 1.7 g/l. Other immunological investigations are shown in Table 3.33.

Table 3.33 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT F.P.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
29. 6.76 2y, 11m Pre T1	84	ND	36.5	0.32	ND	ND
14. 2.77 2y, 3m Pre T1	98	109	0	0.25	ND	ND
30. 5.79 30' Pre T1	95	125	34.9	0.8	5	+
30. 5.79 30' Post T1	95	91	10	0.2	1.2	-
4. 6.79 4 days Post T1	93	94	8	0.5	2.17	-
11. 6.79 11 days Post T1	91	118	18.8	0.16	0.7	-
13. 6.79 13 days Post T1	105	130	41	0.65	3.8	ND
18. 6.79 30' Pre T2	100	120	48	0.4	4.3	-
18. 6.79 30' Post T2	106	108	47	0.5	3	-
22. 6.79 4 days Post T2	115	105	31	0.85	3.2	-
27. 6.79 9 days Post T2	102	106	46.6	0.5	3.8	-
28. 6.79 10 days Post T2	107	110	39.9	0.33	3.8	+
5. 7.79 17 days Post T2	104	46	17.5	0.31	3.6	-
6. 7.79 18 days Post T2	ND	40	40.8	0.31	3.2	ND
9. 7.79 21 days Post T2	96	46	28.7	0.49	0.9	ND
16. 7.79 28 days Post T2	96	100	2.3	0.23	1.5	ND
30. 7.79 1m, 12 days Post T2	ND	ND	4.7	0.25	1.5	ND
21. 3.80 30' Post T3	108	94	4.6	0.33	0	ND
25. 3.80 4 days Post T3	111	98	36.1	0.65	5.9	±
21. 4.80 1m, Post T3	103	100	ND	0.5	4.4	+

Cross-reaction results: The donors: (1) A.P.(brother),(2) L.G. (sister), (3) C.J. (sister).

a. The cross-reactions between the patient and the donors by RIA are shown in Table 3.34.

Table 3.34 CROSS-REACTIONS BY RIA:

	F.P.	A.P.
A.P.	0.55	0.9
L.G.	-ve	0.9
C.J.	0.3	-ve
J.B.K. (normal)	-ve	-ve

b. The cross-reactions between the patient and the donors by the delayed technique are shown in Table 3.35.

Table 3.35 CROSS-REACTIONS BY DELAYED TECHNIQUE:

F.P.	A.P.	L.G.	
1.64	2.05	2.52	alone
F.P.	2.18	2.55	mixture
	1.72	1.81	expected

Results of treatment:

Before the infusion, red painful rashes were distributed on the dorsum of the feet and the patient was not able to walk easily. The rashes began to disappear after the first infusion (30.5.79) but a few small spots were still present and the patient started to walk freely. She lost her IgG complexes immediately after the first infusion (Fig.3.13). The spots disappeared completely 2 days after the second infusion (18.6.79). IgG in circulating complexes appeared within 4 days post second infusion and then disappeared within 9 days. CH50 fell by 17 days post second infusion but later recovered (Fig.3.13). The patient remained in complete remission for 3 months and prednisolone was stopped during this period. She relapsed after 3 months and did not respond to third infusion.

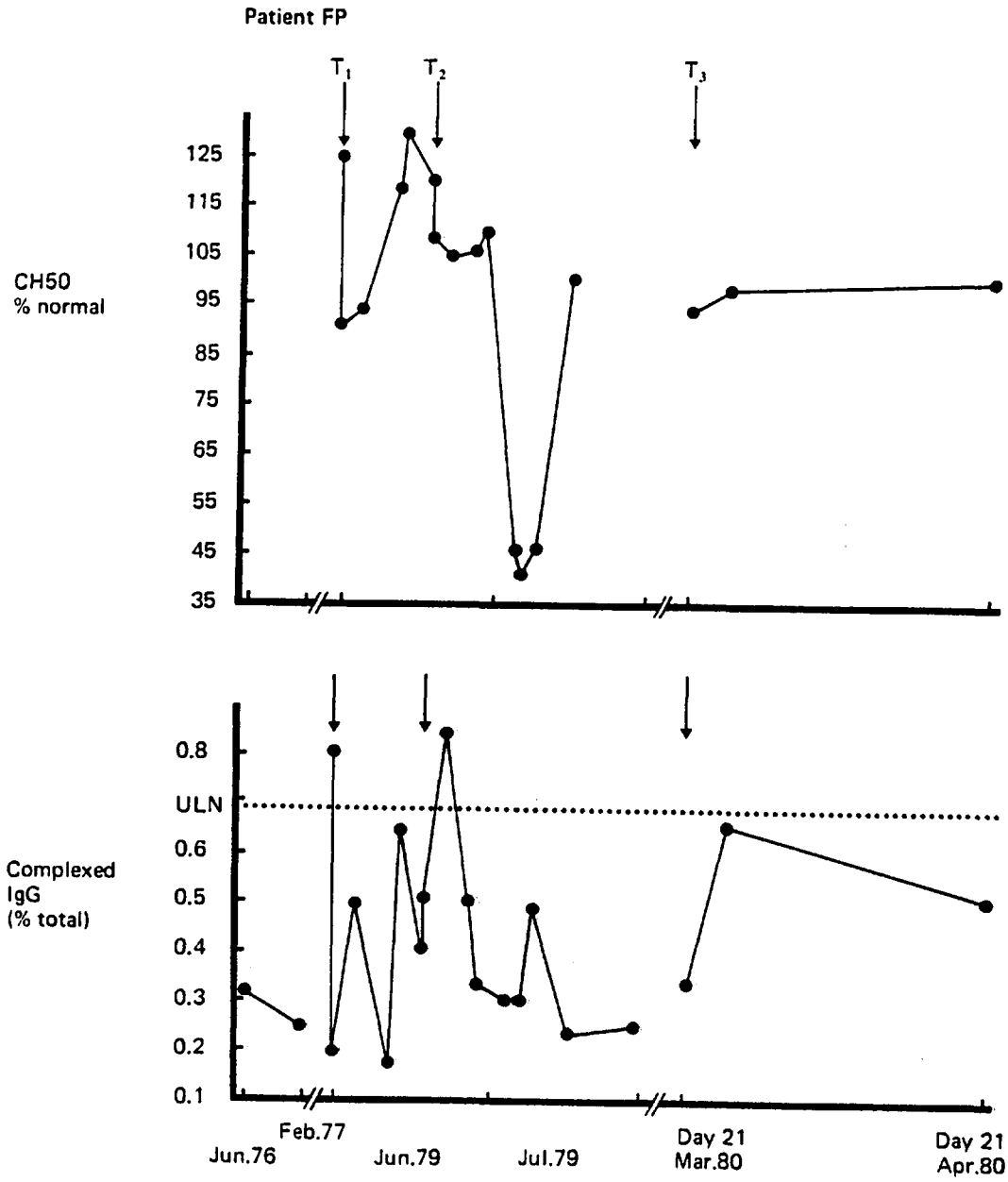


Fig 3.13
CH50 and complexed IgG before and after treatment

Patient K.R. Case 14

This 40 year old lady had had cutaneous vasculitis since 1970. She was admitted to St. Mary's Hospital in 1973 for skin biopsy, and polyarteritis nodosa was diagnosed. She had needed hospitalization on several occasions for the acute attacks of her illness, e.g. fatigue, pyrexia, polyarthritis and painful ulcers over her feet and legs. She continued to have several attacks a year. She was treated with prednisolone to which dapsone and azathioprine were added. The patient was infused on 2 occasions with plasma from her husband (J.R.)(on 8.3.79 and 10.10.79) from one unit each time.

On examination:

All systems were normal. Ulcers or evidence of healing ulcers could be seen on both legs.

Investigations:

WBC 6300/mm^3, Hb 11.6 g/100 ml, ESR 29, kidney function tests and liver function tests normal, total plasma protein 63 g/l, albumin 42 g/l, globulin 21 g/l, IgG 4.7 g/l, IgM 0.17 g/l, IgA 0.6 g/l, ANF negative, rheumatoid factor negative, Australia antigen negative, LE cells negative, MSU negative. Other immunological investigations are shown in Table 3.36.

Table 3.36 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT K.R.		% normal		PEG Precipitable (%)			
date		C3	CH50	C1q	IgG	IgM	IgA
21. 8.78	6m, 16 days Pre T1	120	120	35	0.35	33.6	-
13. 2.79	25 days Pre T1	106	99	5	0.63	8.08	-
8. 3.79	12 hours Post T1	98	112	0	0.18	1.5	-
9. 3.79	1 day Post T1	79	120	0	0.17	1.5	-
12. 3.79	4 days Post T1	93	120	0	1.38	1.5	+
23. 3.79	15 days Post T1	102	110	1.5	0.3	ND	-
18. 4.79	1m, 10 days Post T1	88	130	10.2	0.31	1.5	-
7. 9.79	1m, 3 days Pre T2	87	118	8.9	0.3	0	-
21. 9.79	19 days Pre T2	93	122	3.3	0.19	0.9	-
11.10.79	1 day Post T2	104	126	3.4	0.16	0	ND
16.10.79	6 days Post T2	110	124	20.9	0.16	0.8	-
1.11.79	21 days Post T2	104	116	20	0.2	0	-
14.12.79	2m, 4 days Post T2	101	122	42.7	0.43	0	-

Cross-reaction results: The donor J.R. (husband).

1. The cross-reactions between the patient and the donor by RIA are shown in Table 3.37.

Table 3.37 CROSS-REACTIONS BY RIA:

	K.R.	J.R.
K.R.	2.5	-ve
J.R.	2.5	-ve
MP(normal)	-ve	-ve

2. The cross-reaction between the patient and the donor by the delayed technique is shown in Table 3.38.

Table 3.38 CROSS-REACTION BY DELAYED TECHNIQUE:

K.R.	J.R.	mixture	expected
0.22	1.6	1.8	0.5

Results of treatment:

Before the infusion red painful ulcers were distributed on her feet. The ulcers disappeared completely after the first infusion (8.3.79), and the patient claimed "to feel better". She remained in complete remission until 1.10.79. Prednisolone was stopped during this period. IgG in circulating complexes were found by 4 days post infusion and then disappeared by 15 days. She lost her IgM complexes after infusion (Fig. 3.14). She relapsed after 26 weeks and was treated with another unit of plasma (19.10.79), this induced complete remission, and at the time of writing, 16 months later, she had not relapsed.

Patient H.Z. Case 15

This 60 year old Iranian businessman was admitted to Wellington Hospital in 1979 for further investigations of proteinuria, ill health and fatigue. He was diabetic and treated with oral antidiabetics. Chronic active hepatitis, cutaneous and renal vasculitis were diagnosed. He was infused with 2 units of plasma from his daughter (E.Z.) and son (M.Z.) in April 1979.

On examination:

RS, CVS and CNS were normal. The liver was enlarged. Purpuric spots were present on his trunk.

Results of treatment:

Vasculitis was completely cleared 2 days after the infusion and proteinuria fell to traces which rose again on the fifth day of the infusion, it seems that plasma infusion may have induced a short period of remission. He was lost to follow-up from 10 days after the infusion.

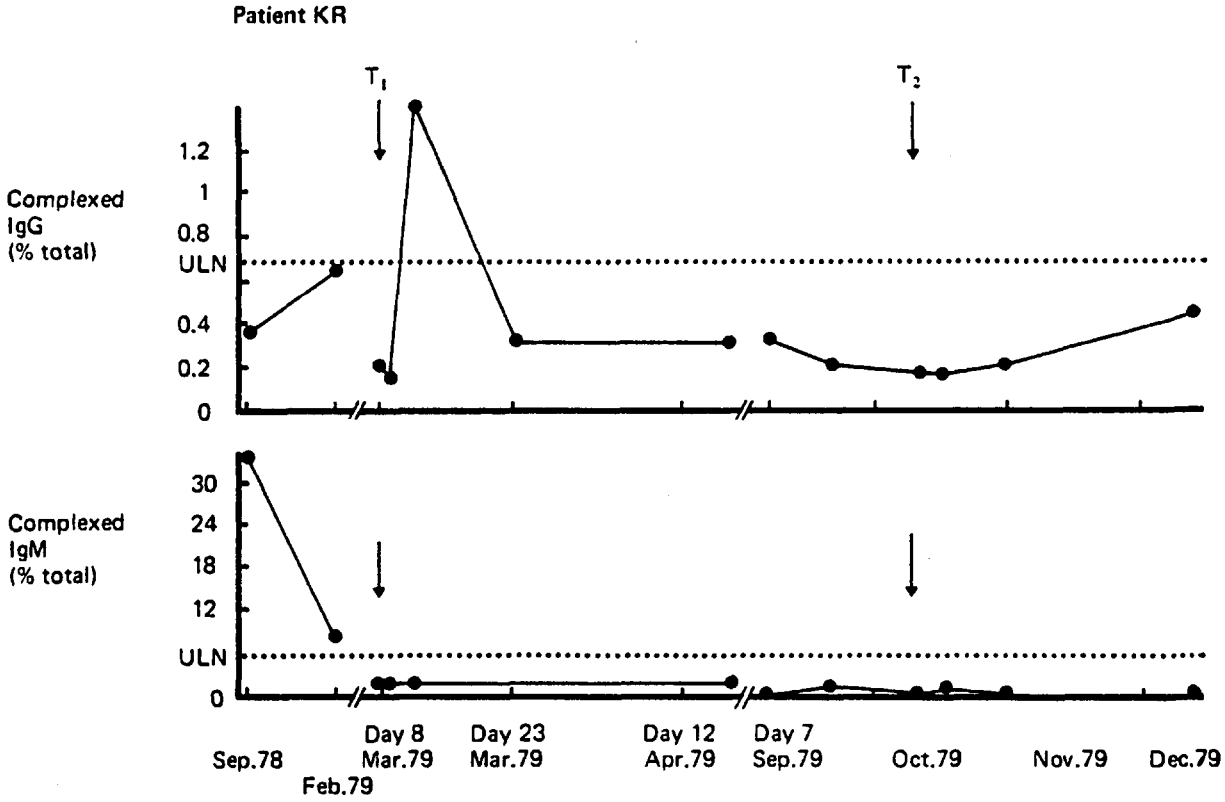


Fig 3.14
Circulating complexes before and after treatment

Patient D.B. Case 16

This 37 year old lady was admitted to U.C.H. on 21.8.78 for renal biopsy complaining of 3 months heamaturia with recurrent ankle swelling. The patient was infused with 1 pint of plasma from her husband (N.B.) on 9.4.79.

On examination:

RS, CNS and CVS were normal. BP 120/80 mm Hg. Some oedema of the feet was noted.

Investigations:

WBC 7300/mm^3, Hb 11.7 g/100 ml, ESR 47, plasma urea 4.8 mmol/l, plasma creatinine 118 µmol/l, plasma protein 55 g/l, albumin 33 g/l, globulin 22 g/l, IgG 3 g/l, IgA 1.8 g/l, IgM 3.7 g/l, serum cholesterol 10.1 mmol/l, ANF negative, DNA binding 7 units/ml, Australia antigen negative, rheumatoid factor negative, MSU sterile but contained moderate numbers of RBC. 24 Hour urine protein excretion was variable between 2.6 and 8 g, IVP was normal, renal biopsy showed focal segmental proliferative glomerulonephritis. Other immunological investigations are shown in Table 3.39. The kidney function of the patient began to deteriorate gradually, her creatinine clearance was 34 ml/min on 21.6.79 and 11 ml/min on 28.7.80.

Table 3.39 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT D.B.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
3. 8.78 8m, 6 days Pre T	101	106	78	0.34	5.3	-
22. 8.78 7m, 17 days Pre T	83	104	16.6	0.29	3.5	-
9.10.78 6m, Pre T	100	110	26.5	0.4	4.4	-
11.10.78 5m, 28 days Pre T	97	56	18	0.83	6.7	-
13.12.78 3m, 26 days Pre T	98	130	29.6	0.3	7.9	-
26. 1.79 2m, 13 days Pre T	87	96	15.8	0.14	9.7	-
28. 2.79 1m, 11 days Pre T	94	88	15.9	0.1	9.7	-
6. 3.79 1m, 3 days Pre T	ND	ND	63	0.38	4.4	-
20. 3.79 19 days Pre T	39	99	58	0.6	12.5	-
1. 4.79 8 days Pre T	73	40	40.2	0.79	10	-
9. 4.79 1 hour Pre T	86	70	40	0.64	8.9	-
11. 4.79 2 days Post T	113	60	60	0.6	11.2	-
23. 4.79 14 days Post T	78	88	4.5	0.22	1.1	-
10. 5.79 1m, 1 day Post T	77	82	34	0.5	5	-
13. 5.79 1m, 4 days Post T	99	90	10.2	0.35	3.9	-
31. 5.79 1m, 22 days Post T	99	119	8.8	0.21	2.7	-
5. 7.79 2m, 26 days Post T	80	0	2.3	0.20	3.9	-
23. 7.79 3m, 14 days Post T	82	114	7.1	0.51	11.7	-
14. 4.80 1y, 5 days Post T	98	80	21.2	0.86	19.7	±

Cross-reaction results: The donors: (1) N.B.(husband), (2) E.S.(sister).

a. Table 3.40 shows the results of the cross-reactions between the patient and the donors by RIA.

Table 3.40 CROSS-REACTIONS BY RIA:

	D.B.	N.B.	E.S.
D.B.	0.3	-ve	-ve
N.B.	0.8	-ve	-ve
E.S.	0.4	0.6	2.5
AS(normal)	-ve	-ve	-ve

b. Tables 3.41 and 3.42 show the results of the cross-reactions between the patient and the donors by the delayed technique.

Table 3.41 CROSS-REACTION BY DELAYED TECHNIQUE:

D.B.	N.B.	mixture	expected
1.03	1.4	1.07	1.104

Table 3.42 CROSS-REACTION BY DELAYED TECHNIQUE:

D.B.	E.S.	mixture	expected
1.8	2.27	1.94	1.89

These cross-reactions in Tables 3.41 and 3.42 were performed on two different occasions.

Results of treatment:

Plasma infusion did not alter the patient's kidney function which still deteriorated and there was no clinical benefit from the treatment. She lost her IgM complexes after the infusion for 3 months and CH50 returned to normal levels.

Patient K.G. Case 17

This 61 year old lady was admitted to U.C.H. on 3.11.75 for renal biopsy. She was discovered to have proteinuria eight months before admission. In the past she had had a splenectomy for easy bruising when aged 15 years which cured her completely.

The patient was infused with 1 pint of plasma from her sister (F.P.) on 23.4.79.

On examination:

RS, CNS and CVS were normal. BP 170/90 mm Hg.

Investigations:

WBC 9200/mm^3, Hb 12 g/100 ml, serum urea 58 mg/100 ml, creatinine 1.5 mg/100 ml, total plasma protein 7 g/100 ml, albumin 3.5 g/100 ml, globulin 3.5 g/100 ml, IgG 1140 mg%, IgA 195 mg%, IgM 81 mg%, rheumatoid slide latex negative, Australia antigen negative, ANF negative, DNA binding 16 units/ml, MSU negative for bacteria but contained moderate numbers of RBC, 24 hour urine protein excretion 2.1 g. The last kidney function tests before the infusion were; creatine clearance 7 ml/min, plasma urea 24.8 mmol/l, plasma creatinine 635 µmol/l. Renal biopsy showed focal glomerulonephritis. Other immunological investigations are shown in Table 3.43.

Table 3.43 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT K.G.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
3.10.77 1y, 7m Pre T	101	105	44	0.4	ND	-
30. 1.78 1y, 3m Pre T	106	114	66.5	0.76	ND	-
20. 3.78 1y, 1m Pre T	95	121	67	0.62	ND	-
9.10.78 6m, 14 days Pre T	109	96	33	0.63	ND	-
13.12 78 3m, 10 days Pre T	105	91	48.9	0.48	1.04	-
9. 4.79 14 days Pre T	85	100	75.9	0.9	3.4	±
23. 4.79 30 minutes Pre T	93	120	49.3	0.44	0.4	-
23. 4.79 30 minutes Post T	93	130	35.2	0.5	1.3	±
26. 4.79 3 days Post T	106	135	5.1	0.48	1.3	-
28. 4.79 5 days Post T	98	90	24.4	0.78	1.3	-
3. 5.79 10 days Post T	100	135	17.3	0.42	1.3	-
10. 5.79 17 days Post T	87	79	5.1	0.48	1.3	-
21. 5.79 28 days Post T	101	120	19.9	0.43	1	-
31. 5.79 1m, 8 days Post T	100	116	18.8	0.23	1.8	-
9. 7.79 2m, 16 days Post T	107	26	25.8	0.25	0	ND
23. 7.79 3m, Post T	90	120	7.1	0.45	0	ND

Cross-reaction results: The donors:(1) F.P.(sister, (2) E.W.(sister), (3) D.T.(sister).

a. The cross-reactions between the patient and the donors by RIA are shown in Table 3.44.

Table 3.44 CROSS-REACTIONS BY RIA:

	F.P.	D.T.	E.W.	J.B.(normal)
K.G.	0.6	-ve	-ve	-ve

N.B. - K.G. is the labelled sample

b. The cross-reactions between the patient and the donors by the delayed technique are shown in Table 3.45.

Table 3.45 CROSS-REACTIONS BY DELAYED TECHNIQUE:

K.G.	F.P.	D.T.	E.W.	
2.42	3.5	2.6	3.07	alone
	2.56	1.62	4.16	mixture
	2.63	2.46	2.55	expected

Results of treatment:

There was no clinical improvement in the patient and her kidney function stayed the same, e.g. the creatinine clearance after the infusion was 6 ml/min and the plasma urea 23 mmol/l. IgG in the circulating complexes appeared by 5 days post infusion and then disappeared by 10 days.

Patient S.A.H. Case 18

This 22 year old Bangladeshi kitchen porter was admitted to U.C.H. in 1977 for investigations of heavy proteinuria. For six months before admission he had had polyuria and nocturia. The only obtainable history was that in 1971 he had had typhoid fever and jaundice. He was treated with diuretics and high protein diet. On 3.4.79 he was infused with 1 pint of plasma from his father (E.H.).

On examination:

All systems were normal. Blood pressure was 120/70 mm Hg. There was no ankle oedema.

Investigations:

WBC 8.8 x 10^9/L, RBC 49 x 10^{12}/l, ESR 48, Hb 12.4 g/100 ml, blood urea 6.6 mmol/l, plasma creatine 97 µmol/l, cholesterol 7.8 mmol/l, ANF negative, DNA binding 20 units/ml, plasma protein 55 g/l, albumin 32 g/l, globulin 23 g/l, IgG 7.8 g/l, IgA 1.1 g/l, IgM 2.6 g/l. MSU was sterile on several occasions, no pus cells were found but haematuria was moderate. 24 Hour urine protein excretion on different occasions was 13 g, 12 g. Other immunological investigations are shown in Table3.46. IVP showed that both kidneys were enlarged with poor contrast concentration, a finding compatible with a nephrotic syndrome. Renal biopsy showed mesangiocapillary glomerulonephritis.

Table 3.46 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT S.A.H.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
17.11.77 1y, 5m Pre T	100	72	28	0.48	ND	ND
2. 2.78 1y, 2m Pre T	103	100	25	0.5	4.5	-
12. 4.78 1y, 9 days Pre T	102	86	43	0.2	6.2	-
11.10.78 5m, 22 days Pre T	112	104	54	0.43	4.3	-
12. 3.79 21 days Pre T	93	90	12.3	0.38	3.6	-
5. 4.79 2 days Post T	107	106	36.7	0.33	4.9	+
9. 4.79 6 days Post T	68	68	49.3	0.54	7	-
17. 4.79 14 days Post T	100	30	26.4	1.2	3.2	-
23. 4.79 20 days Post T	98	30	5.1	0.5	1.3	-
30.4.79 27 days Post T	86	92	10.2	0.35	1.5	-
14. 5.79 1m, 11 days Post T	93	90	7.1	0.38	3.6	-

Cross-reaction results: The donor: E.H. (father).

1. The cross-reaction between the patient and the donor by RIA was negative.
2. The cross-reaction between the patient and the donor by the delayed technique was negative and is shown in Table 3.47.

Table 3.47 CROSS-REACTION BY DELAYED TECHNIQUE:

S.A.H.	E.H.	mixture	expected
0.92	2.2	1.1	1.36

Results of treatment:

The patient showed no clinical improvement after the infusion and there was no change in the amount of proteinuria, which we consider as a titre for the improvement. IgG in circulating complexes were found by 14 days post infusion, but disappeared by 20 days. IgM complexes were found by 6 days post infusion then disappeared. C3 and CH50 fell by 6 days post infusion but later recovered.

Patient L.P. Case 19

This 53 year old Greek man was admitted to U.C.H. in 1976 for the removal of varicose veins when he was noted to have mild renal failure with heavy proteinuria, he was normotensive but became hypertensive a few months later; he had noticed nocturia 3 times nightly for several months. He was treated with β-blockers and hydrallazine. On 14.5.79 the patient was infused with 1 pint of plasma from his daughter (M.P.).

On examination:

RS, CNS and CVS were normal, there was no heart failure, kidneys were not palpable and there was no renal artery bruit. Oedema was not present. By ophthalmoscopy marked A-V nipping was present but there was no papilloedema. Blood pressure was 200/100 mm Hg.

Investigations:

WBC 7500/mm^3, Hb 15.8 g/100 ml, ESR 23, urea 50 mg/100 ml, plasma creatinine 2.1 mg/100 ml, electrolytes were normal, total plasma protein 7 g/100 ml, albumin 3.8 g/100 ml, globulin 3.2 g/100 ml; creatinine clearance 64 ml/min, Australia antigen negative, ANF negative, 24 hour urine protein excretion 1.9 g, MSU sterile but contained few RBCs. Renal biopsy showed mesangiocapillary glomerulonephephritis. Other immunological investigations are shown in Table 3.48. The last kidney function tests before the infusion were creatinine clearance 21 ml/min, plasma urea 10.9 mmol/l.

Table 3.48 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT L.P.		% normal		PEG Precipitable (%)			
date		C3	CH50	C1q	IgG	IgM	IgA
30. 1.78	1y, 4m Pre T	ND	ND	95	1.3	ND	-
13. 4.78	1y, 1m Pre T	ND	ND	77.5	0.58	3.16	ND
9. 4.79	1m, 5 days Pre T	87	80	75.5	0.6	1.8	+
30. 4.79	14 days Pre T	109	100	38	0.5	0.2	ND
13. 5.79	1 day Pre T	89	130	10.2	0.35	1.5	-
17. 5.79	3 days Post T	95	94	52	0.8	3.4	-
21. 5.79	7 days Post T	95	116	52	1.4	2.4	-
4. 6.79	20 days Post T	120	92	29.7	0.36	1.3	-
14. 6.79	1m Post T	109	100	73.8	0.65	4.4	ND

Cross-reaction results: The donors: (1) M.P.(daughter), (2) J.P.(daughter), (3) M.P. (son), (4) C.P. (son).

1. The cross-reactions between the patient and the donors by RIA were negative.

2. The cross-reactions between the patient and the donors by the delayed technique are shown in Table 3.49.

Table 3.49 CROSS-REACTIONS BY DELAYED TECHNIQUE:

L.P.	M.P.	J.P.	M.P.	C.P.	
1.7	2.05	3.5	1.27	1.64	alone
L.P.	1.54	1.84	1.85	2.16	mixture
	1.66	2.06	1.61	1.68	expected

Results of treatment:

The patient showed no clinical improvement after the infusion and his kidney function stayed the same. IgG in the circulating complexes appeared by 3 days post infusion and then fell by 20 days.

Patient W.W. Case 20

This 56 year old male was admitted to U.C.H. on 22.5.1978 for renal biopsy. Six months prior to admission he was discovered to have hypertension, proteinuria, haematuria and renal failure. He was treated with β-blockers and diuretics. On (8.6.79) the patient was infused with 1 pint of plasma from his daughter (J.W.).

On examination:

He was slightly tired. RS and CVS were normal. BP 195/105 mm Hg. There was slight oedema of his left leg.

Investigations:

WBC 9.5 x 10^9/l, RBC 4.15 x 10^{12}/l, Hb 11.9 g/100 ml, urea 25 mmol/l, plasma creatine 491 μmol/l, total serum protein 68 g/l, albumin 39 g/l, globulin 29 g/l, IgG 10.9 g/l, IgA 3.9 g/l, IgM 1.4 g/l, creatinine clearance 15 ml/min, Australia antigen negative, ANF negative, DNA binding 10 units/ml (within normal limits), syphilis serology negative, MSU sterile but contained moderate numbers of RBC. 24 Hour urine protein excretion was 6.42 g, renal biopsy showed focal proliferative glomerulonephritis. Other immunological investigations are shown in Table 3.50.

Table 3.50 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT W.W.		% normal		PEG Precipitable (%)			
date		C3	CH50	C1q	IgG	IgM	IgA
4. 5.78	1y, 1m Pre T	97	122	14.3	0.18	7.3	-
2. 4.79	2m, 6 days Pre T	86	50	62.8	1.4	11	±
21. 5.79	17 days Pre T	101	120	77	0.81	4.5	±
8. 6.79	1 hour Pre T	127	109	39	0.45	2.7	-
12. 6.79	4 days Post T	108	118	47	0.26	0.5	+
14. 6.79	6 days Post T	109	70	89	1.7	6.2	-
15. 6.79	7 days Post T	98	118	6.1	0.6	3.4	+
18. 6.79	10 days Post T	105	60	5.9	0.85	1.7	+
21. 6.79	13 days Post T	114	50	7.2	1.1	3.8	+

Cross-reaction results: The donors: (1) V.W.(wife), (2) J.W.(daughter), (3) P.W.(son).

a. The cross-reactions between the patient and the donors by RIA are shown in Table 3.51.

Table 3.51 CROSS-REACTIONS BY RIA:

	W.W.	J.W.
W.W.	-ve	0.8
J.W.	0.5	-ve
V.W.	-ve	0.4
P.W.	-ve	-ve
J.F.M.(normal)	-ve	ND
A.S.(normal)	-ve	ND

b. The cross-reactions between the patient and the donors by the delayed technique are shown in Table 3.52.

Table 3.52 CROSS-REACTIONS BY DELAYED TECHNIQUE:

W.W.	J.W.	V.W.	P.M.	
2.27	0.93	1.84	1.14	alone
	2.27	2.05	2.5	mixture
	1.99	2.17	2.13	expected

Results of treatment:

The patient showed no clinical improvement after the infusion and his kidney function stayed the same, e.g. the creatinine clearance after the infusion was 15 ml/min and the plasma urea was 27.2 mmol/l. Circulating immune complexes were found by 6 days post transfusion but disappeared by 7 days, CH50 fell by 6 days post infusion but recovered by 7 days to fall again later.

Patient J.G. Case 21

This 50 year old lady had had chronic pericarditis for several years. No viral studies were positive at any stage of her illness. She was infused with one pint of plasma from her husband (R.G.) on 1.2.80.

Complement and immune complex data are shown in Table 3.53.

Table 3.53 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT J.G.		% normal		PEG Precipitable (%)			
date		C3	CH50	C1q	IgG	IgM	IgA
18. 4.78	1 y, 10 m Pre T	80	86	59	0.52	6.8	-
13.12.79	1m, 18 days Pre T	81	66	66.5	0.53	9.7	-
1. 2.80	30' Pre T	87	86	17.9	0.26	3.96	-
1. 2.80	30' Post T	85	91	14.6	0.24	3.53	-
2. 2.80	1 day Post T	96	75	30.6	0.29	4.41	-
9. 2.80	8 days Post T	92	58	50.3	0.67	5.48	-
28. 2.80	27 days Post T	113	108	42	0.33	11.2	-
19. 3.80	1m, 18 days Post T	84	70	12.1	0.33	2.35	-
6. 5.80	3m, 5 days Post T	101	58	12.1	0.33	0.35	+
20. 6.80	30' Pre inj.	91	55	ND	0.59	5.5	-
20. 6.80	2 hours Post inj.	91	55	ND	0.63	3.96	-
21. 6.80	1 day Post inj.	78	60	ND	0.58	6.75	-

Cross-reaction results: The donors: (1) R.G.(husband), (2) D.G. and E.G.(family members). The cross-reactions between the patient and the donors by the delayed technique are shown in Table 3.54.

Table 3.54 CROSS-REACTIONS BY DELAYED TECHNIQUE:

J.G.	R.G.	D.G.	E.G.	
0.98	1.64	0.98	0.98	alone
J.G.	1.73	0.98	1.52	mixture
	1.11	0.98	0.98	expected

Results of treatment

Two to three days after transfusion the patient felt worse but within one week she claimed "to feel better than she had felt for a very long time" and was in remission for four months. IgM complexes were detected by 27 days post-transfusion and then disappeared by 48 days; CH50 levels fell after transfusion (Fig.3.15). She developed a new mild attack of chest pain in June 1980 which was treated with a single injection of immunoglobulin on 20.6.80. This induced complete remission 10 days later with no recurrence until March (the termination of our studies).

Patient S.H. Case 22

This 50 year old lady had had percarditis and cardiomyopathy for several years. She was treated with immunosuppression and steroids and on one occasion exacerbation of her symptoms was accompanied by the presence of a Coxsackie B-4 neutralizing antibody titre of 1/256. The patient was infused with one unit of plasma from her husband (A.H.) on 13.3.80. Complement and immune complex data are shown in Table 3.55.

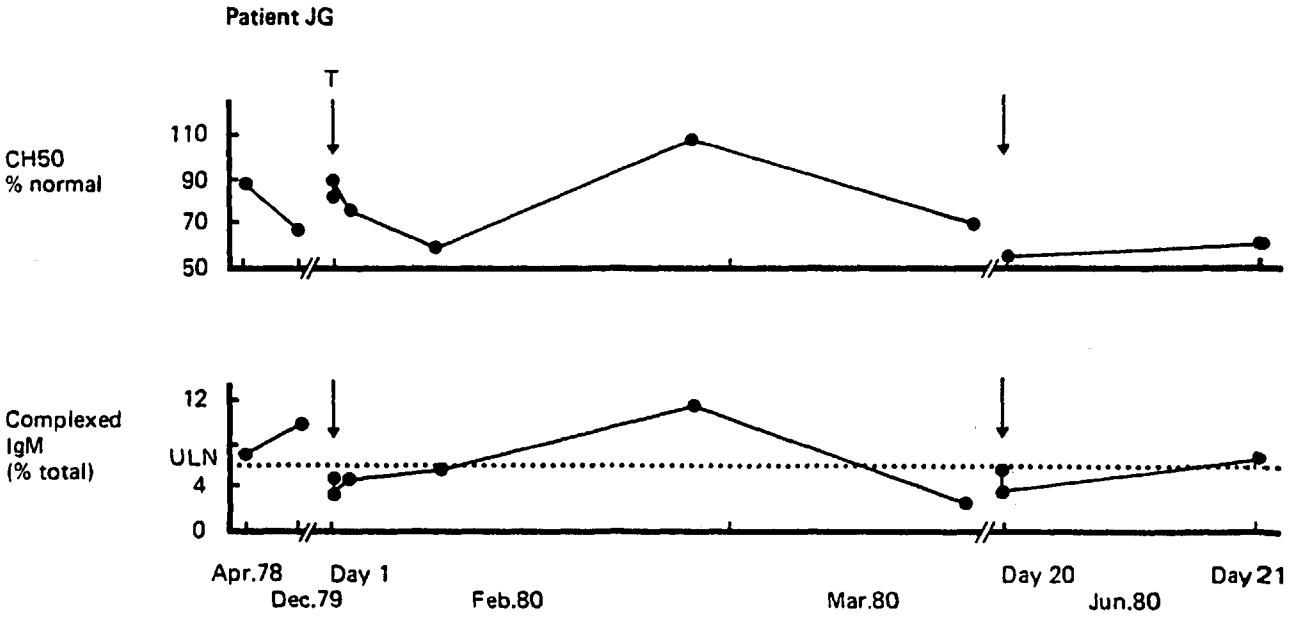


Fig 3.15
CH50 and complexed IgM before and after treatment

Table 3.55 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT S.H.	% normal		PEG Precipitable (%)			
date	C3	CH50	C1q	IgG	IgM	IgA
23. 3.78 2 years Pre T	88	99	56.5	0.84	ND	-
7. 7.78 1y, 8m Pre T	96	110	67	0.46	4.9	+
10. 8.78 1y, 7m Pre T	94	86	54.5	1.01	7.6	+
14. 9.78 1y, 6m Pre T	109	90	19	0.38	6.3	+
19.10.78 1y, 5m Pre T	96	100	79	0.71	2.1	-
8.12.78 1y, 3m Pre T	88	110	25.7	0.52	3.8	-
12. 3.79 1 year Pre T	109	113	100	2.1	5.7	+
23.10.79 4m, 20 days Pre T	93	65	29	0.4	1.8	+
21. 1.80 1m, 22 days Pre T	91	67	27.6	0.53	1.91	+
13. 3.80 30' Pre T	105	107	44	0.76	6.34	+
13. 3.80 30' Post T	97	102	31.6	0.52	5.09	-
14. 3.80 1 day Post T	107	102	30.1	0.4	5.14	±
20. 5.80 2m, 7 days Post T	87	102	ND	0.76	4.18	±

Cross-reaction results: The donors: (1) A.H.(husband), (2) N.H. & J.H. (family members). The cross-reactions between the patient and the donors by the delayed technique are shown in Table 3.56.

Table 3.56 CROSS-REACTIONS BY DELAYED TECHNIQUE

S.H.	A.H.	N.H.	J.H.	
0.7	0.5	1.52	1.15	alone
S.H.	1.5	1.73	1	mixture
	0.66	0.865	0.79	expected

Results of treatment:

There was no clinical benefit from the treatment. She lost her IgG and IgM complexes immediately after the infusion, but IgG complexes appeared by the tenth week (Fig.3.16).

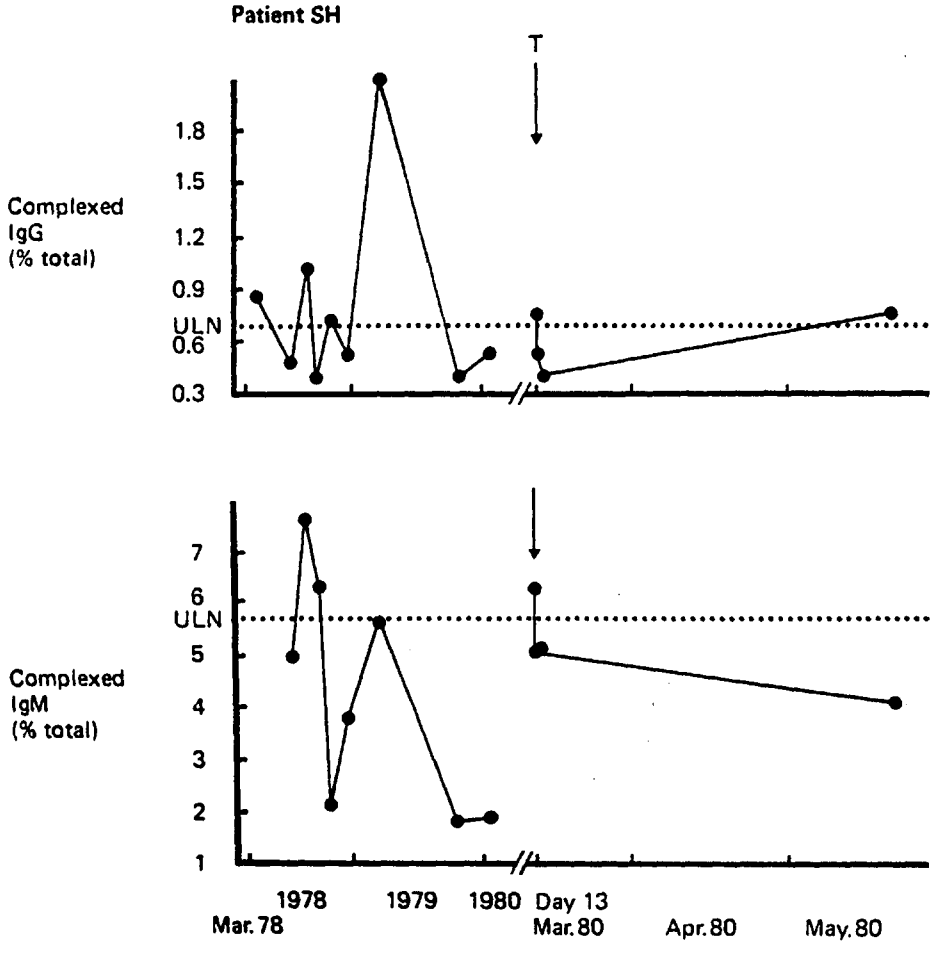


Fig 3.16
Circulating complexes before and after treatment

Patient M.S. Case 23

This 35 year old lady had had chronic pericarditis since 1976. She was treated with intramuscular injections of immunoglobulin on two occasions (on 19.6.80 and 13.2.81). Complement and immune complex data are shown in Table 3.57.

Table 3.57 COMPLEMENT AND IC DATA BEFORE AND AFTER GAMMA GLOBULIN INJECTION.

PATIENT M.S.		% normal		PEG Precipitable (%)			
date		C3	CH50	C1q	IgG	IgM	IgA
30.12.76	3y, 6m Pre inj.	88	79	9.2	0.19	ND	-
4. 2.77	3y, 5m Pre inj. 1	96	75	9	0.09	ND	+
7. 3.78	2y, 3m Pre inj. 1	89	91	10.4	0.1	ND	-
2. 2.79	1y, 5m Pre inj. 1	88	76	19	0.78	ND	+
11. 2.80	4m, 8 days Pre inj.1	108	76	66.4	0.54	4.5	-
19. 6.80	1 hour Pre inj. 1	95	64	ND	ND	5.91	+
19. 6.80	3 hours Post inj.1	102	66	ND	0.74	5.1	±
20. 6.80	1 day Post inj. 1	81	55	ND	0.65	4.34	±
27. 6.80	8 days Post inj. 1	94	84	ND	0.72	5.5	-
3. 7.80	14 days Post inj.1	91	76	ND	0.65	6.15	±
18. 7.80	29 days Post inj.1	92	108	ND	0.75	6.86	±
12. 2.81	1 day Pre inj. 2	96	70	53	0.59	5.9	±
14. 2.81	1 day Post inj. 2	96	90	58	0.41	3.6	-
23. 2.81	10 days Post inj.2	95	77	60	0.42	6.53	±
2. 3.81	19 days Post inj.2	101	116	45	0.34	6.85	±

Results of treatment

Ten days after injection (19.6.80) the patient felt better and went into complete remission for 6.5 months. CH50 rose by 8 days post injection. IgG complexes remained high and she lost her IgM complexes for 14 days (Fig. 3.17). The patient was treated with another injection of immunoglobulin (on 13.2.81) which induced new remission

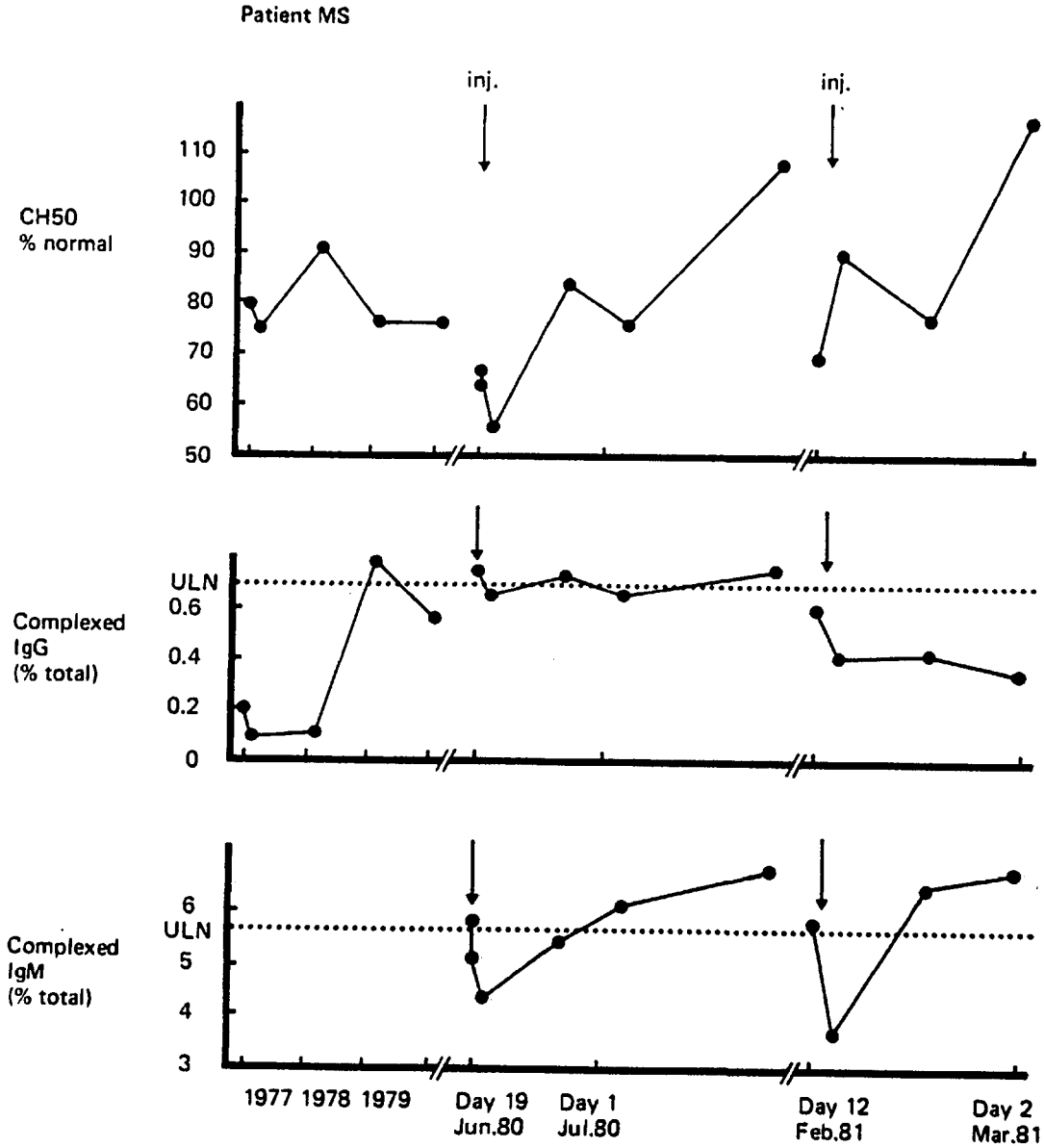


Fig 3.17
CH50 and circulating complexes before and after treatment

Patient J.W. Case 24

This 46 year old lady had had chronic pericarditis since 1978. This was characterized by episodes of chest pain. The patient was infused with 1 unit of plasma from her husband (G.W.)(on 23.5.80). Complement and immune complex data are shown in Table 3.58.

Table 3.58 COMPLEMENT AND IC DATA BEFORE AND AFTER TRANSFUSION

PATIENT J.W.		% normal		PEG Precipitable (%)			
date		C3	CH50	C1q	IgG	IgM	IgA
6. 7.79	10m, 17 days Pre T	95	81	66.7	0.57	7.5	±
13. 8.79	9m, 10 days Pre T	100	94	23.2	0.35	8.21	-
23.11.79	6 months Pre T	106	112	96.9	1.06	10.2	-
8. 2.80	3m, 15 days Pre T	125	106	58.3	0.4	4.98	±
12. 3.80	2m, 11 days Pre T	108	104	7.7	0.26	0	-
22. 5.80	1 day Pre T	100	76	ND	0.27	1.4	-
23. 5.80	30' Pre T	111	76	ND	0.4	2.66	-
23. 5.80	30' Post T	101	78	ND	0.27	2.34	-
24. 5.80	1 day Post T	107	84	ND	0.17	2.34	-
29. 5.80	6 days Post T	107	108	ND	0.33	2.93	-
12. 6.80	18 days Post T	92	75	ND	0.49	6.44	-
19. 6.80	25 days Post T	111	90	ND	0.87	8.50	-
25. 6.80	1m, 1 day Post T	81	84	ND	0.3	4.46	-
27. 6.80	1m, 3 days Post T	107	90	ND	0.62	6.64	+
11. 8.80	2m, 18 days Post T	94	80	57	0.34	7.8	-
28. 8.80	3m, 5 days Post T	100	75	36.1	0.39	3.80	-
12. 1.81	7m, 19 days Post T	117	120	38	0.37	7.39	-

Cross-reaction results: The donor: G.W. (husband). The cross-reaction between the patient and the donor by the delayed technique is shown in Table 3.59.

Table 3.59 CROSS-REACTION BY DELAYED TECHNIQUE

J.W.	G.W.	mixture	expected
1.51	2.5	1.59	1.7

Results of treatment

The patient was treated with one pint of plasma. She was in complete remission a few days after the infusion, and at the time of writing, 9 months later she had not relapsed. CH50 rose after infusion. IgG in circulating complexes appeared by 25 days post infusion but then disappeared. IgM complexes were detected by 18 days and then disappeared by 1 month (Fig. 3.18).

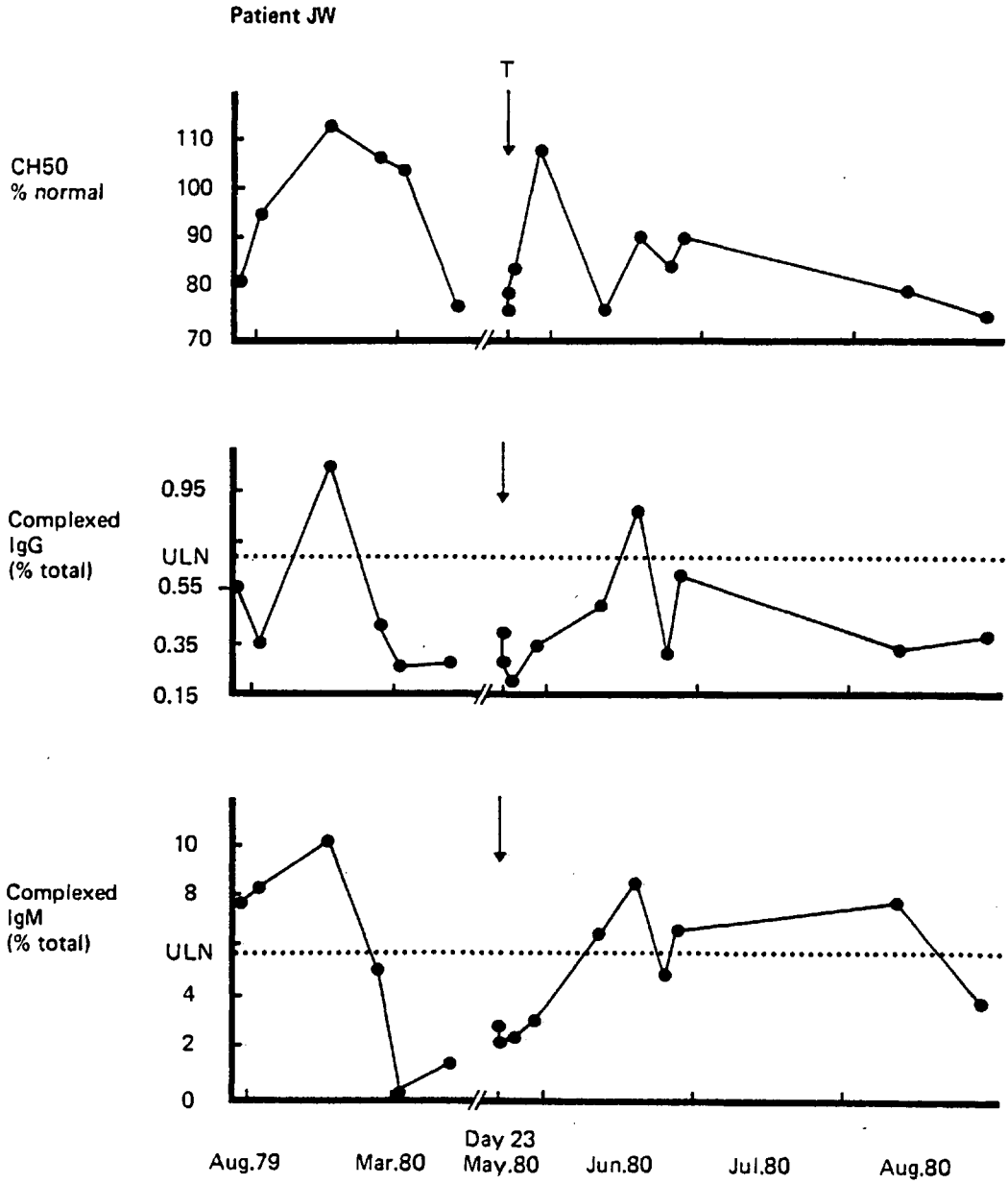


Fig 3.18
CH50 and circulating complexes before and after treatment

List of tables and figures summarizing types of treatment and results obtained

a. Table 3.60 shows the results and type of treatment in all patients

b. Table 3.61 shows the maximum length of remission and the fraction of time (months) spent in remission of 21 patients before and after treatment (plasma and/or gamma globulin). For details of the type of treatment see Table 3.60.

c. Fig. 3.19 shows the maximum length of remission (months) before and after treatment.

d. Fig. 3.20 shows the fractional period of remission (months in remission/ total months of disease) before and after treatment.

N.B. The cross-reactions in Tables 3.9, 3.12, 3.14, 3.16, 3.32, 3.38, 3.41, 3.54, 3.56 and 3.59 were done by Mrs. D. Zewdie.

Table 3.60 RESULTS AND TYPES OF TREATMENT IN ALL PATIENTS

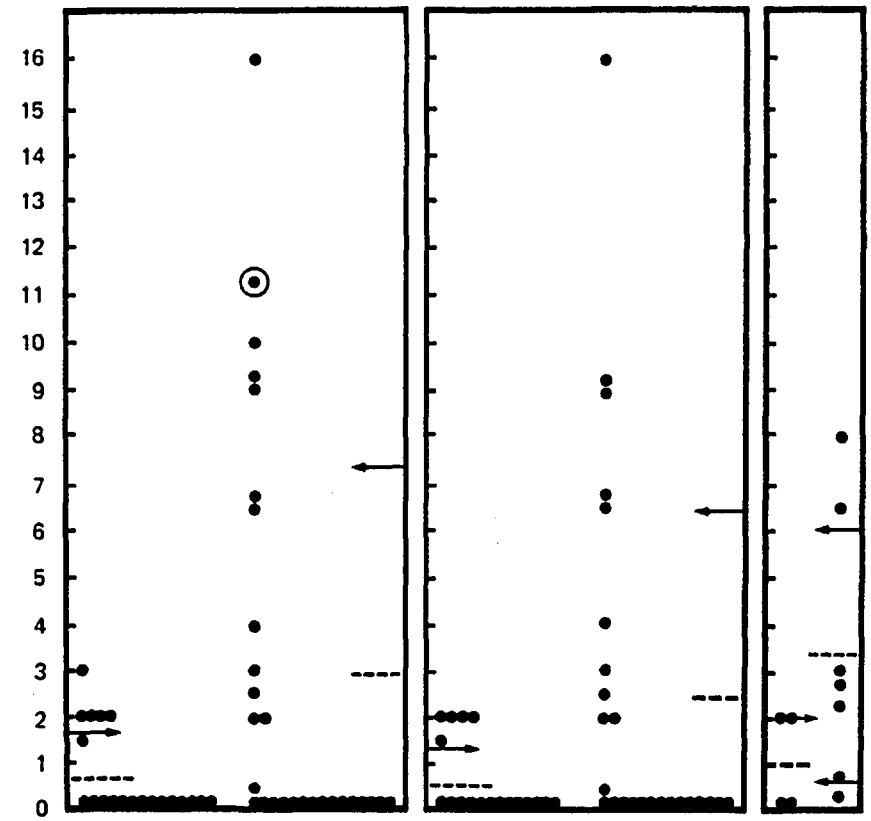
Patient	Circulating complexes	Type of treatment	Remission (months)
S.G.	IgG, IgM	PF1	2
		PF2	2
		G1	2.75
		G2	0.75
T.H.	IgM	PF	6.75
		G1	2.25
		G2	3
M.O.	IgG, IgM	PF	9.25
J.S.	C1q, IgM	FFP	0
F.A.	C1q, IgG	6PP (PPF)	2
		3PP (FFP)	2.5
		6PP (FFP)	2
F.M.	C1q, IgG, IgM	B	11.25
	IgA	PF1	2.5
		PF2	0
W.M.	C1q, IgG	5PP (PPF)	1
		4PP (FFP)	3
F.B.	C1q, IgG	PF	0
I.J.	C1q, IgG, IgM	FFP	10
R.J.	C1q, IgG, IgM, IgA	PF	0
F.L.	C1q, IgG, IgA	PF	0
R.M.	C1q, IgG, IgM, IgA	PF1	0
		PF2	0
		PF3	0
F.P.	IgG, IgA	PF1	0.5
		PF2	3
		PF3	0
K.R.	C1q, IgG, IgM	PF1	6.5
		PF2	16
H.Z.	ND	PF	0.25
D.B.	C1q, IgG, IgM	PF	0
K.G.	C1q, IgG	PF	0
S.A.H.	IgG, IgM	PF	0
L.P.	C1q, IgG	PF	0
W.W.	C1q, IgG, IgM, IgA	PF	0
J.G.	C1q, IgM	PF	4
		G	8
S.H.	C1q, IgG, IgM, IgA	PF	0
M.S.	C1q, IgG, IgM, IgA	G1	6.5
		G2	0.3
J.W.	C1q, IgG, IgM, IgA	PF	9

N.B. - PF = Plasma (from the) Family; G = Gamma globulin; FFP = Fresh Frozen Plasma; PP = Plasma Pheresis; PPF = Plasma Protein Fraction; B = Blood; ND = Not Done.

Table 3.61 MAXIMUM LENGTH AND FRACTIONAL PERIOD OF REMISSION BEFORE AND AFTER TREATMENT.

Patient	Db	Da	Rb	Ra	Max.Rb	Max.Ra	B	A
S.G.	40	2.47 2.6 3.13 1	0	2 2 2.75 0.75	0	2 2 2.75 0.75	0	0.809 0.769 0.878 0.75
T.H.	28	7.14 2.8 3.26	0	6.75 2.25 3	0	6.75 2.25 3	0	0.945 0.803 0.92
M.O.	18	10	12	9.25	2	9.25	0.666	0.925
J.S.	84	4	63	0	3	0	0.75	0
F.M.	1	14.3 3.37 4	0	11.25 2.5 0	0	11.25 2.5 0	0	0.786 0.741 0
F.B.	60	4	0	0	0	0	0	0
I.J.	37	14	0	10	0	10	0	0.714
R.J.	139	4	0	0	0	0	0	0
F.L.	144	4	0	0	0	0	0	0
R.M.	104	1.86 0.76 4	6	0 0 0	1.5	0 0 0	0.057	0 0 0
F.P.	55	0.6 9.1 2	2	0.5 3 0	2	0.5 3 0	0.036	0.833 0.329 0
K.R.	105	7 16.23	0	6.5 16	0	6.5 16	0	0.928 0.985
D.B.	11	4	0	0	0	0	0	0
K.G.	49	4	0	0	0	0	0	0
S.A.H.	23	4	0	0	0	0	0	0
L.P.	39	4	0	0	0	0	0	0
W.W.	19	4	0	0	0	0	0	0
J.G.	176	4.63 8.37	nd	4 8	2	4 8	nd	0.863 0.955
S.H.	98	4	0	0	0	0	0	0
M.S.	50	7.8 1	nd	6.5 0.3	2	6.5 0.3	nd	0.833 0.3
J.W.	23	9.26	3.5	9	2	9	0.152	0.972

N.B. – Db = length of disease before treatment (months)
Da = length of disease after treatment (months)
Rb = Remission before treatment (months)
Ra = Remission after treatment (months)
Max.Rb = Maximum Rb
Max.Rb = Maximum Ra
B = fraction of Rb
A = fraction of Ra
nd = No Date



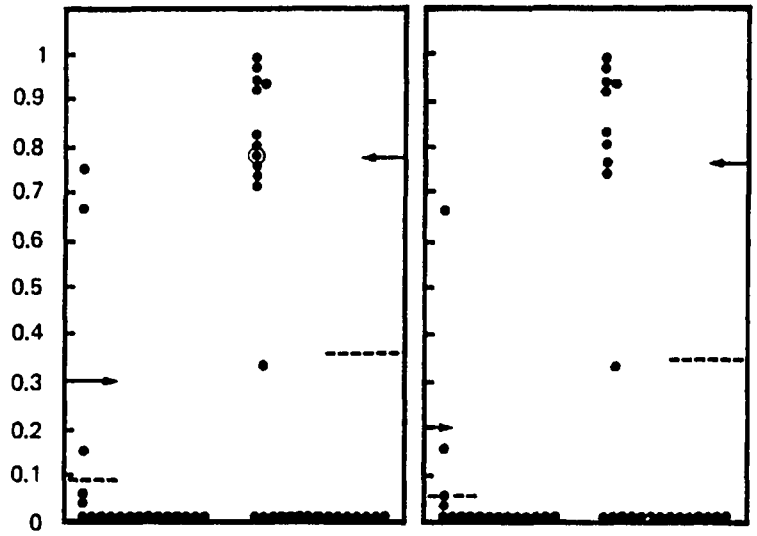
	Before	After	Before	After	Before	After
Mean	0.625	2.955	0.527	2.46	1	3.364
S.D.	0.985	4.35	0.857	3.992	1	2.653
n	20	28	18	25	4	7
	t = 2.348	2P < 0.05	t = 2.015	2P ≃ 0.05	t = 1.1683	2P > 0.05

Patients treated with plasma including blood. ⊙ =blood

Patients treated with familial plasma.

Patients treated with gamma globulin.

Fig 3.19
Maximum length of remission before and after treatment (months)



	Before	After	Before	After
Mean	0.087	0.36	0.053	0.343
S.D.	0.216	0.418	0.157	0.423
n	19	27	17	24
	t = 2.625	2P ≃ 0.01	t = 2.710	2P < 0.01

Patients treated with plasma including blood. ⊙ = blood

Patients treated with familial plasma.

Fig 3.20
Fractional period of remission before and after treatment

CHAPTER FOUR
DISCUSSION

Composition of Immune Complexes in Infectious Diseases

The most important observation from the immune complex analysis data is the large number of IgM complexes with few IgG complexes in sera from patients with chronic pericarditis (which is possibly due to viral infection). These striking changes may reflect the immune competence of these patients. I shall discuss below the possible causes of the large number of IgM containing complexes and their subsequent effects on these patients. This reveals a possible reason for the disease chronicity.

1. Defect in IgM-IgG switch mechanism and antibody affinity:

IgM is the earliest antibody response to a primary immune stimulus, followed after a short interval by IgG antibody which is usually formed as the secondary response to an antigen stimulus (van Oss, 1979). The maturation of an immune response towards an antigenic stimulus, in order to achieve successful elimination of this antigen, seems to require a change in both the antibody class and affinity. It is known that IgG antibody which is produced in response to viral infection continues to be synthesised for many years and confers protection against viral infection for life, while the IgM titre declines within weeks of viral infection (Fenner and White, 1976; Schmidt et al, 1968; Bellanti, 1978). This switch mechanism usually requires helper T cells to be present and the defect in this mechanism may be due to a defect in helper T cells which co-operate with B cells in antibody formation (usually IgG class) (Paul and Benacerraf, 1977). Thus, a deficit in T cells may impair the IgM-IgG switch mechanism and the production of memory cells. These cells are produced during the primary exposure to an antigen and the secondary response depends on their existence (Herscowitz, 1978). Therefore repeated antigenic challenges with the same or a different antigen would stimulate a primary response

(IgM antibody), with subsequent incomplete clearance of the antigen.

Soothill and Steward (1971) suggested that a failure to produce high affinity antibody to an antigen may be due to a defect in macrophages or helper-cells processing an antigen, and might predispose to the development of immune complex disease (the effects of high affinity antibodies on the patients are discussed later). Thus, the failure to produce a proper antibody might be accompanied by incomplete elimination of infection, allowing long lasting antigen production; which might lead to chronic disease or chronic carrier.

2. T-independent antigens:

Certain antigens, e.g. pneumococcus polysaccharides, endotoxin or polyvinyl pyrrolidine, are able to react with antibody on the surface of a B cell and stimulate it independently of T-cell help. Such an antibody is usually of IgM class (Paul and Benacerraf, 1977; Roitt, 1980). It is possible that the antigens present in the circulating immune complex (IgM-antigen) of the sera from the studied patients are of T-independent type. But if some people produce IgG and others IgM, then the antigen is not T-independent in all people. Thus it would appear more likely to be different in the patients rather than the antigen.

3. Early presentation:

It is possible that some patients present themselves for investigation during the primary immune response and before the normal IgM-IgG switch mechanism, which usually occurs about two weeks after the initiation of the immune response (Herscowitz, 1978).

4. Non-neutralizing antibodies:

Viruses can persist and multiply in macrophages and lymphocytes for many years. These viruses can stimulate the production of non-neutralizing antibodies (perhaps of IgM class?). The produced antibodies may combine with viral antigens to form immune complexes, which may (a) block immune

cytolysis of virus-infected cells by T lymphocytes or complement-fixing antibodies and, (b) produce "immune complex disease" (Fenner and White, 1976).

5. Presence of more than one antigen:

Tied in with the previous point is the possibility that the presence of multiple antigens with different immunogenicities may induce continuous production of two types of antibody, e.g. IgM and IgG. If IgM-antigen complex is formed it may block the production and the function of other immunoglobulins, e.g. IgG. This early low affinity antibody might cover the virus and protect it from the neutralising effect of high affinity. In time, however, the high affinity antibody should attach itself by displacing the low affinity antibody.

6. Immunodepression by virus:

Viral infection can impair immunological host responsiveness (Turk, 1978). It has been shown that mice infected with coxsackie virus developed reduced humoral and cellular immune responses to simultaneous infection with poliovirus (Bendinelli et al, 1975). The investigators however did not characterize the class of antibody produced.

7. Genetic (HLA system):

The association between HLA and susceptibility to certain disease, e.g. HLA B27 and ankylosing spondylitis (Brewerton et al, 1973) has been a matter of extensive investigation in recent years. A possible explanation for this association is that HLA antigen may play a receptor role for a virus which can induce a certain disease (Morris, 1979). A second explanation is that the host's sharing antigens with infecting organisms may produce a poor immune response and a weak antibody in the early phase of the disease, perhaps of IgM class, with some antiself-activity (Young et al, 1978). This concept might explain the

chronicity due to a persistent low antibody response or the autoantibody and its effect (Morris, 1979; Silver et al, 1972).

8. Tolerance:

Chronic viral infections in experimental animals may be associated with a poor antibody response and persistent tolerant infection especially in neonatal or congenitally infected animals with virus, e.g. lymphochoriomeningitis virus (LCM)(Hotchin and Collins, 1964; Fenner and White, 1976). Notkins et al (1966) have shown persistent infectious circulating immune complexin the blood of mice chronically infected with LDH virus. This may be due to production of poorly neutralizing antibody. Such tolerance seems to parallel the carriage of human serum hepatitis, where persistence of infection in asymptomatic Australia antigenaemia is found without an antibody response (Fenner and White, 1976).

9. Antigenic variation:

I have mentioned previously that parasites can change their antigenic structure during infection and hence escape from the effective host immune response. Can viruses do the same? Antigenic variation in influenza virus is common, and responsible for new influenza pandemics, but if it happens in an individual, an antibody to the new antigen would be released (Jawetz et al, 1978). It, therefore, does not seem an important point.

10. Immunosuppressive treatment:

It has been shown experimentally that mice develop fatal infections with Coxsackie B-3 virus when they have been treated with an immunosuppressive agent (cyclophosphamide)(Rager-Zisman and Alison, 1973). This treatment allowed transient IgM production but inhibited IgG production in those mice. A number of the patients in these studies were treated with immunosuppressive agents during the course of their illness, but it is

not possible to assess from our records if this treatment affected their immune response, although the disease has always preceded the immunosuppressive treatment. There is no clear evidence however that all patients with IgM complexes were treated with immunosuppressive agents. Two other observations arise from the immune complex assay data:

1. The increase in the amount of "IgA-only" containing complexes in pericarditis sera rather than in other pathological sera. This occurs when the infection is through the mucous surface, but may also occur for some other unknown reason.
2. The amount of C1q in pericarditis complexes is less than in other pathological sera. One possible reason for this is that the antibodies involved are non-complement fixing. It should be remembered however that pericarditis is not an immune complex disease.

These points might explain the pathogenesis of certain chronic diseases especially those due to infection, and may suggest that viral infection plays a role in these diseases. It seems that it is important to bear in mind the facts which arose from the experimental work that the class and affinity of antibodies are of a great importance for complete and adequate elimination of infection (Rager-Zisman and Allison, 1973; Alpers et al, 1972). These facts might help us to find more effective treatments of certain diseases which are immunologically mediated.

Cross-Reaction by Radioimmunoassay

I have mentioned that the aim of this assay was to provide further evidence of the presence of mutual causal agent(s) in a group of patients with similar disease by studying the antigenic cross-reactions between their circulating immune complexes. It has enabled us to study sera from several diseases in which there are circulating immune complexes. It was also used to study the cross reaction between the donor's serum and the patient's immune complex in an attempt to choose a proper donor and to see if it was possible to predict which donor's plasma would cause remission. It was apparent from the cross-reaction results in certain disease groups, e.g. dermatomyositis (no cross-reaction), SLE (36% total + ve reactions), Tb (16.2% total + ve reactions), pericarditis (8.7% total + ve reactions), that cross-reactions either did not occur at all or only to a small degree by this assay, as one might expect to find more positive reactions than we found. In comparison with other disease groups the number of positive cross-reactions were very low, e.g. hypertension/haematuria (90.6% total + ve reactions). I shall discuss the possible reasons for this low number of positive reactions:

1. Acid dissociation of antigen-antibody complex:

In our radioimmunoassay, antigen-antibody complex solutions were dissociated in glycine-HCl solution pH 3.1. This pH tends to make all protein components in the solution positively charged which causes the antigen-antibody complexes to loose their ability to attract each other (van Oss and Grossberg, 1979). The acid dissociation may affect IgM complexes since it has been found that IgM complexes are acid labile and they could be destroyed under acid conditions (Mowbray and Abdalla unpublished data). Another possible disadvantage of this

procedure is that acid dissociation may affect acid labile antigen(s) in the solution. However the use of higher pH for the dissociation of immune complexes may overcome this for certain acid labile antigens but this may reduce the dissociation efficiency (Dambuyant et al, 1979).

2. The iodination procedure:

Immune complexes in our assay were labelled using the chloramine T method (Dambuyant et al, 1979) a modification of Hunter's methods (1973) by which iodine reacts with tyrosine residues of the protein molecule. This method can cause certain damage to the protein, e.g. radiation damage or chemical damage (Hunter, 1973) which may change the antigen and antibody reactivity and decrease the number of positive reactions.

3. Antigen/antibody ratio:

It has been shown by this technique that a higher binding of radioactive material was noted with soluble complexes obtained in antigen excess and a lower reactivity of soluble complexes obtained in antibody excess (Dambuyant et al, 1979). This may cause negative binding results, in antibody excess conditions.

4. PEG precipitation:

I shall talk briefly about the PEG and its effect on immune complexes. Immune complexes in sera used in the radioimmunoassay were precipitated with polyethylene glycol (PEG). PEG is a polysaccharide (polymer) which in solution can precipitate serum proteins according to their molecular weight and proportional to the concentration of PEG (Zubler et al, 1977; Digeon et al, 1977). Therefore at low concentrations of PEG high molecular weight proteins and immune complexes are precipitated. Other factors which affect the IC precipitation are the pH (Riha et al, 1979) and the temperature of the solution (Digeon et al, 1977).

The exact mechanism of PEG precipitation of immune complexes at this low concentration is unknown, but it is possible that the protein-polymer combination may mask the surface properties which govern protein solubility (Harrington et al, 1971).

From the previous observations about the cross-reactions by radioimmunoassay it is important to emphasize that negative results do not necessarily mean that cross-reactions do not occur, or that there is no common causal agent in these diseases.

This study may help in the future to identify the antigens which are responsible for the immune complex disease and their elimination by proper therapy.

Rheumatoid Factor Binding Assay

The aim of the rheumatoid factor binding assay was to study the ability of the donor's antibodies to react with the patient's circulatory complexes by measuring the ability of this mixture (donor/patient sera) to bind the rheumatoid factor preparation which was labelled with ^{125}Iodine. This was done in an attempt to choose a proper donor for plasma infusion (similar in principle to the other cross reaction methods between the patient and the donor which was mentioned earlier in this chapter). It was obvious from the cross-reaction results by this assay that the binding values of ^{125}I-RF to complexes were low and this assay was not a successful cross-reaction assay.

Summarized below are a number of reasons as to why this may not be a good procedure:

1. Iodination procedure: (discussed previously)

   Iodine can cause certain damage to the protein molecule.

2. Interference factors:

   Intrinsic rheumatoid factor and complement complicate the results and may give false positive results (Thompson, 1978). Other colleagues started to standardize the method and tried to overcome some of the above problems, for example:

   a) Pre-assay heat inactivation of C1q by 56°C incubation for 30 minutes of the tested sera.

   b) Adding normal horse serum to the tested sera in an attempt to block the intrinsic rheumatoid factor.

These changes did not increase the percentage bound and the assay in general did not improve. These low values might be explained by the fact that the rheumatoid factor used may have had a low activity for some other reason, in particular, IgG RF is possibly not a good detector of multiple Fc interactions. Perhaps it would have been better

to use a monoclonal IgM RF (Barratt and Naish, 1979). The increased affinity of RF for complexes is dependent on the multiple bonds to Fc regions of a complex. Comparing IgM RF with IgG RF it is obvious that IgM RF is potentially more capable of distinguishing circulating complexes than IgG RF, due to the multivalency of the former.

The Effects of Treatment in the Patients Studied

The group of patients studied was initially as wide as possible and before I discuss the effects of treatment (plasma and/or gamma globulin) I shall discuss briefly the nature of the diseases of the patients who were treated. These diseases can be divided into two groups:

I. Immune complex diseases:

In this group, the diseases are usually associated with the deposition of immune complex either generally or locally. Examples of generalized diseases in our series are systemic lupus erythematosus, rheumatoid arthritis, glomerulonephritis and polyarteritis nodosa (possibly).

The role of immune complexes in some of these diseases has been discussed previously.

An example of localized disease in our series is erythema multiforme.

Erythema multiforme is an acute inflammatory skin disorder. It usually begins as a widespread erythematous skin lesion (reddening of the skin), and involves the forearms, hands, and legs. This may go on to bulla formation (Rees, 1980). It may be associated with virus infection, e.g. herpes simplex virus (Shelley, 1967), drug eruptions or is of unknown origin (Rees, 1980). In three of our four patients with erythema multiforme, herpes simplex preceded the acute attacks of erythematous rashes.

The aetiologic agent(s) remains unknown or elusive in the majority of immune complex diseases but the following observations may suggest that infection may play a role in some immune complex diseases, e.g. SLE and PAN.

1. Beaucher et al (1977) reported that in families with related patients with SLE, dogs with SLE were also found.

2. The presence of C-type viral antigen-antibody complexes in kidneys of patients with lupus nephropathy (Panem et al, 1978).
3. The presence of Australia antigen in sera of patients with PAN (Trepo et al, 1974).

II. Non-immune complex disease: e.g. pericarditis

Pericarditis is an inflammation of the pericardium and may occur as a primary disorder which is probably due to viral infection, mainly Coxsackie virus, or as a part of general disease, e.g. tuberculosis and rheumatic fever (Julian and Matthews, 1977). It can present acutely with subsequent recovery or chronically with remissions and relapses, Recurrence may be due to autoimmune or other mechanisms (Sokolow, 1980).

I have mentioned in the first part of the discussion some possibilities which can be used to explain different aspects of the chronicitiy of this disease. The role of immune complexes in this disease is uncertain and the presence of circulating immune complexes is probably due to the patient's normal response and does not necessarily indicate a causal relationship. Our laboratory data show that about 68% of sera from patients with pericarditis contains circulating immune complexes.

We have treated patients with percarditis and myocarditis which are probably due to viral infection.

Twenty-one of the patients studied were treated initially with plasma infusion, either fresh frozen plasma or plasma from their close relatives (including spouse) and one patient (M.S.) was treated only with intramuscular injections of immunoglobulin.

The possible effects of plasma infusion followed by intramuscular injections of gamma globulin in some patients are discussed.

The possible uses and effects of plasma infusion:

1. Plasma as a source of antibodies:
   a. High affinity antibodies.
   b. Protective antibodies to infectious antigens.
   c. Changing immunoglobulin class.
   d. Prophylactic use.
2. Replacement of complement components.
3. Removal of immune complexes.
4. Inhibition or removal of the inflammatory mediators.

1. Plasma as a source of antibodies

a. High affinity antibodies

It is believed in this concept that the addition of immune serum containing high affinity antibodies may help to clear antigen more efficiently and protect against certain diseases. This belief arises from the following observations:

Soothill and Steward (1971) have shown that mice prone to glomerulonephritis from neonatal infection with lymphatic choriomeningitis virus, produce low affinity antibody in response to immunisation with human serum albumin or transferrin, while the nephritis-resistant strains produce higher concentration of antibody of higher affinity. Alpers et al (1972) reported that the rateof elimination of antigens in different strains of mice is attributed to the affinity of the antibody they produce, and those mice that form high affinity antibody are more capable of eliminating antigen than those producing low affinity antibody. Pincus et al (1968) reported that rabbits which produce non-precipitating antibody in response to repeated injections of bovine serum albumin showed an increased liability to develop chronic nephritis.

It is likely that the remission observed in our patients after plasma infusion, from near relatives (including spouse) in the majority of cases or fresh frozen plasma in a few cases, was due to the addition of

immune serum containing specific antibody probably with high affinity.

I have mentioned that viral infection may play a role in the pathogenesis of the diseases of the patients whom we treated. If such is the situation, then this makes environmental infections a possible aetiology in many of our patients. It is likely that the unaffected members of the family (usually the donors) may handle the infection differently from the patient and may produce high affinity antibody to the same agent to which the patient may be unable to produce an effective antibody response. Therefore the remission which had resulted in some of those patients might be due to this type of antibody which was found in the infused plasma. And it is likely that those donors who are exposed to infection could be used as a source of highly effective antibody. Also this type of effective antibody might be found in gamma globulin prepared from pooled human serum.

This concept was supported by an important observation in some of our patients, e.g. K.R. and F.P., as after effective infusion the patient's serum produced IgG complexes. This leads to the proposition that those patients may be unable to make IgG antibody to the agent to which the donors can. Delire and Masson (1977) successfully treated with gamma globulin children with recurrent upper RT infections of unknown aetiology. Circulating immune complexes were detected in 30 out of their 39 patients. Our findings and those of Delire and Masson (1977) make it likely that some patients, with immune complex disease or recurrent infections, have a deficiency of a specific antibody, and transfusion or injection of this antibody may result in long term remission possibly because more effective handling of the infectious agent may occur.

b. Protective antibodies to infectious antigens

Antibodies to any antigenic determinant of an infectious agent do not always confer immunity. For example, neutralization of viral infectivity is due mainly to antibodies against the surface antigens of

the virus, while antibodies directed against the internal component of the virus do not neutralize viral activity (Fenner and White, 1976). In orthomyxoviral infection resistance to initiation of infection is related to antibodies to the haemagglutinin portion of the virus. The non-neutralizing antibodies may combine with viral antigens and form immune complexes which may result in immune complex diseases (Fenner and White, 1976; Jawetz et al, 1978). Webster and Laver (1967) reported that antibody directed to a surface antigen, the haemagglutinin, of an influenza virus neutralized haemagglutinin activity and infectivity while antibody against neuraminidase did not inhibit haemagglutinin activity of the virus. The previous observations may suggest that a specific deficiency of antibody to the surface viral antigen in the patient would allow the virus infection to be a persistent source of antigens to produce immune complex disease. It is possible that the addition of a neutralizing antibody to the surface antigen might clear the infection and allow the elimination of the source of the antigen with accompanying remission of the disease. And as has been mentioned in the previous paragraph a suitable source of this type of antibody might be found in the plasma of people who are exposed to the same agent in the same environment, or possibly, if the agent is common in the population, in some other sources such as immunoglobulins prepared from pooled human serum.

c. Changing immunoglobulin class

i. IgM to IgG:

By plasma infusion we may replace IgM by IgG. This may lead to more effective handling of infection, since IgG provides a mechanism for killer or K-cell cytotoxicity. These killer cells have cytotoxic activity with target cells, including virus-infected cells coated with specific IgG antibody in an antibody dependent cellular cytotoxic reaction (Bellanti, 1978; MacLennan and Harding, 1974).

ii. IgE to IgG:

As mentioned, the IgE molecule plays an important role in immediate hypersensitivity reactions. The interaction of an antigen with IgE molecules on the surface of the mast cell or basophil triggers the release of vasoactive amines. These substances could be important in the localization of immune complexes (Roitt, 1980). In this concept it is presumed that if IgG antibodies are injected into the body they can combine with antigen and prevent it from reacting with IgE molecules. This may inhibit the production of vasoactive amines from mast cells and prevent the localization of immune complex (WHO Scientific Group, 1976). This type of therapy (giving IgG to compete with the antigen and prevent the reaction with IgE) has been successfully used in the treatment of allergic individuals (Thompson, 1978). However the role of this phenomenon in our treatment is unknown.

d. Prophylactic use

It is possible to use plasma and/or gamma globulin injection to prevent recurrent infection and subsequent disease (Miller, 1973). To achieve this the infusion needs to be performed at intervals. In some of our patients effective plasma infusion was followed by intramuscular injection of gamma globulin either to induce a new remission or to protect the patient from a severe attack of the disease, as in patients T.H. and S.G.

There are several advantage of plasma infusion over intramuscular injections of immunoglobulin:

i. Antibodies of all immunoglobulin classes (IgG, IgM, IgA, IgE, IgD) can be supplied in a large amounts by plasma infusion (Buckley, 1977).

ii. Plasma infusion provides higher serum immunoglobulin concentrations than intramuscular immunoglobulin injections (Buckley, 1977), for example, each 100 ml of plasma contains 1 g of IgG.

iii. Intravenous plasma infusions are less painful than intramuscular injections of immunoglobulins (Buckley, 1977).

It is important to remember, however, the difficulties in comparing the effects of these two treatments in our patients because by plasma infusion we give multiple agents.

Some other uses of immunoglobulin:

There are other specific indications for the clinical uses of immunoglobulin:

i. Primary immunodeficiency states

In primary hypogammaglobulinaemia, e.g. Bruton's disease, immunoglobulin therapy seems to be highly effective (Janeway and Rosen, 1966; Bruton, 1952).

ii. Secondary hypogammaglobulinaemia

Immunoglobulin levels decrease secondary to certain primary disorders e.g. severe protein loss, chronic lymphatic leukaemia or myeloma (Roitt, 1980; Hayward, 1977). Immunoglobulin therapy is indicated in secondary hypoglobulinaemia when IgG levels are low (less than 2 grams/litre)(Miller, 1973). However as soon as the basic disorders are corrected this therapy can be stopped.

iii. Prophylaxis of viral and bacterial diseases

There are a number of indications for immunoglobulin therapy to prevent the occurrence of several infectious diseases, e.g. measles, hepatitis A, poliomyelitis, chicken pox, Lassa fever, tetanus, pertussis and complications of mumps (hyperimmune gamma globulin is required in the last four conditions)(Janeway and Rosen, 1966; Kabat, 1963; Fenner and White, 1976). This is because suitable gamma globulin which may lead to more effective handling of these infrequent infections is usually found in the serum of hyperimmune persons.

2. Replacement of complement components

I have described previously the fact that the complement system is a group of serum proteins which plays an important role in the host's defence against infections. They may be activated in two ways, the classical pathway and the alternative pathway.

The most important purposes of complement investigations are, firstly, that the detection of complement components may be useful to monitor the results of treatment. Thus return of the complement values to normal levels during treatment in certain diseases, e.g. SLE could be used as an indicator of remission (Mowbray, 1977). Secondly, the detection of complement components may be useful to determine the course of the disease, e.g. persistent hypocomplementaemia in patients with acute post-streptococcal glomerulonephritis may indicate rapidly progressive disease (Baldwin, 1977).

In recent years increasing attention has been directed towards congenital deficiencies affecting the complement system. It has been found that inherited defects of complement components may predispose to immune complex disease. The role of complement deficiencies in disease has been discussed by many investigators. I shall review briefly some of the literature concerning this matter.

Friend et al (1975) reported a patient with C2 deficiency with chronic vasculitis and a history suggesting increased liability to infection. Day et al (1973) reported systemic lupus erythematosus and membranous glomerulonephritis in a patient with congenital C2 deficiency. Alper et al (1972) reported homozygous deficiency of C3 in a patient with repeated infections. Systemic lupus erythematosus-like disease and glomerulonephritis have been observed in a patient with C1r deficiency (Day et al, 1972). Peters et al (1973) suggested that the presence of hypocomplementaemia in mesangiocapillary glomerulonephritis, due to C3 activation, may result in immune complex nephritis by predisposing the patient to recurrent infection. These observations led to the suggestion that those patients with complement deficiency have an abnormality of the immune responses resulting in immune complex disease (Peters and Lachmann, 1974).

Since it is known that complement components are provided by plasma infusion one might ask whether the remission in some of our patients was due to complement replacement and whether their improvement was due to restoration of the depleted serum complement components in them. The answer is not very clear as we do not have evidence of congenital or acquired complement deficiency in our patients, except for those with systemic lupus erythematosus. Two of them (F.A. and W.M.) were treated with plasmapheresis, and the only longstanding improvement occurred when the patients' plasma were replaced with fresh frozen plasma rather than the albumin containing plasma protein fraction (PPF)(Moran et al, 1977). This may suggest that the improvement in those two patients might be due to complement replacement. Other patients in our series went into a long-term remission after infusion, lasting for months, making it unlikely that the improvement was due to complement replacement in them, the half-life of complement components being measured in hours, not months (Ruddy et al, 1972).

3. Removal of immune complexes

Immune complexes are formed when antigens interact with their corresponding antibodies. The formation of immune complexes is a component of normal immune responses. They are usually removed from the circulation by phagocytosis. When immune complexes are deposited in the tissue they may cause immune complex disease (Carter, 1973; WHO Scientific Group, 1976; Kohler, 1973).

It has been reported that circulating immune complexes are correlated with the clinical disease activity in a group of patients with immune complex diseases, for example systemic lupus erythematosus infective endocarditis, and Henoch-Schönlein purpura. Sustained reduction in their circulating complexes after treatment may be associated with clinical improvement (Levinsky et al, 1977; Pussell et al, 1978; Mowbray and Burton, 1976). This suggests that the improvement in the

patients with the above diseases might be due to the removal of the immune complex or its source.

There is good evidence that plasmapheresis can produce successful results in some patients with immune complex diseases. This may be due to the removal of these complexes or of antigens or antibodies or to the addition of high affinity antibody or to some other reasons (Pinchinig, 1980; Jones et al, 1976; Lockwood et al, 1976).

Plasmapheresis has been successfully used in the treatment of Goodpasture's syndrome, by removing nephrotoxic antibodies (Lockwood et al, 1975, 1976). Also it has been used to treat systemic lupus erythematosus. Jones et al (1976) reported a striking clinical and immunochemical improvement in patients with SLE who have been treated with plasmapheresis. Chalopin et al (1980) have reported recovery after plasmapheresis in a patient with polyarteritis nodosa associated with HBs antigen. One might ask whether plasma infusion induces successful effects similar to those of plasmapheresis?

In our method of plasma infusions with small volumes (usually one unit of plasma) the quantity was much smaller than those used in plasma exchange centres. The effect of these small infusions might be determined from our results in which it is obvious that treatment was highly effective in some patients and independent of the volume of the exchange.

Thus one pint of plasma or less is required to alter the host's immune balance; in some patients a single injection of gamma globulin was enough to induce remission for months.

It is possible that plasma infusion may remove immune complexes from the circulation which could deposit and cause an immune complex disease by the following mechanisms:

a. Alteration of antigen-antibody ratio

Alteration of the antigen-antibody ratio may change the properties of complexes, e.g. size, and enhance their elimination. It has been

shown that large complexes are rapidly removed by the hepatic reticulo-endothelial system while small complexes are not (Mannik and Arend, 1971). Levinsky et al (1977) showed that extrarenal manifestations in patients with SLE-nephritis were associated with large IgG complexes, whereas in those with renal manifestations medium sized IgG complexes were found. This leads us to assume that the addition of extrinsic antibody which is found in the infused plasma or some other sources, e.g. the injected immunoglobulin, may be able to compete with or to displace the intrinsic complex antibody for antigen and might change the properties of immune complex and enhance its elimination.

b. The production of C3b

Certain enzymes (such as plasmin, thrombin, trypsin, $C\overline{42}$, $C\overline{3bBb}$ and cobra venom factor) are known to be capable of splitting C3. This splitting yields C3a and C3b (Lachmann, 1975). The latter, together with immune complexes, binds to specific sites on polymorphs and macrophages and produces the immune adherence phenomenon. This phenomenon enhances phagocytosis of immune complexes and other particles, e.g. bacteria or viruses (Roitt, 1980; Ruddy et al, 1972). Thus after infusion, if the patient's plasma can activate the donor's C3 (which yields C3b), then the immune adherence phenomenon may occur in the patient with subsequent removal of the immune complex.

In some of our patients the clinical improvement after plasma infusion was accompanied by reduction of circulating immune complexes (of patients F.P. and I.J.), while in other patients (D.B., F.B., S.H. and R.J.) this reduction was not accompanied by any clinical improvement.

The possible results of immune complex removal are:

i. Removal of low affinity antibody:

Low affinity antibody competes with antigen and prevents it from combining with high affinity antibody.

ii. Reduction of the load of immune complexes on the reticuloendothelial system (RES).

iii. Stimulation of antibody forming cells:

It has been shown experimentally that the removal of serum antibody by exchange transfusion from guinea pigs previously immunised with sheep red blood cells stimulates the proliferation of 19 S antibody forming cells in their spleen (Strugill and Worzniak, 1970). Can plasma infusion induce the same effect in man?

4. Inhibition or removal of inflammatory mediators

a - Platelets

Immune complex can aggregate platelets and lead to the release of phospholipid procoagulant and vasoactive amines which participate in tissue injury (WHO Scientific Group, 1976; Roitt, 1980). Immune complex elimination by plasma infusion may stop this mechanism and prevent the production of harmful substances. The exact role however and importance of platelets in certain human disease, e.g. nephritis is not yet clear (Peters, 1979).

b - Leukocytes

Immune complex elimination may inhibit the stimulation of leukocytes and prevent the release of the destructive enzymes from these cells which participate in tissue injury (WHO Scientific Group, 1976; Leaf and Cotran, 1980).

c - Mast cells

Mast cells are responsible for the release of vasoactive amines when they are activated and degranulated through the interaction of antigen-antibody (usually IgE-antigen)(Roitt, 1980; Bellanti, 1978). If we prevent the release of these harmful substances by blocking mast cells one might expect to arrest the damage and inhibit the inflammation process. This might be achieved after plasma infusion by changing

Ig class (e.g. IgE to IgG)(discussed previously), which blocks the FC receptor on these cells without degranulation (WHO Scientific Group, 1976).

Statistical evaluation of the plasma infusion treatment

In order to evaluate the benefit to the patients in terms of remission, the effect of plasma infusion treatment has been assessed statistically. Therefore, both the maximum length of remission and the proportion of time spent in remission (months), before and after treatment, were compared using a Student's t-test. Significant improvement was judged to have occurred when the mean remission after treatment was significantly elevated ($2P \leq 0.05$) above that found before treatment.

A total of twenty-eight plasma treatments (including blood) were performed on twenty patients. The maximum length of remission was measured for each treatment and compared to that found before treatment. The mean ($\pm$ S.D.) maximum remissions before and after treatment were 0.625 ± 0.985 and 2.955 ± 4.35 months respectively. This difference was found to be statistically associated with the treatment ($t = 2.348$, $2P < 0.05$). Of these twenty-eight plasma treatments, twenty-five were performed on eighteen patients using plasma obtained from family members (including spouse). In these latter cases, mean ($\pm$ S.D.) remission was also greater after treatment (2.46 ± 3.992) compared to the pre-treatment values (0.527 ± 0.857, $t = 2.015$, $2P \simeq 0.05$).

Similar results were obtained from a statistical treatment of the fractional period of remission (months in remission/total months of disease) before and after plasma treatment (including blood) for a total of twenty-seven observations on nineteen patients, mean ($\pm$ S.D.) fractional remissions before and after treatment were 0.087 ± 0.216 and 0.36 ± 0.418 respectively ($t = 2.625$, $2P \simeq 0.01$). Of these twenty-seven observations, twenty-four were performed on seventeen patients using plasma obtained from family members (including spouse).

In these latter observations, mean (± S.D.) fractional remission was also greater after treatment (0.343 ± 0.423 compared to pre-treatment values (0.053 ± 0.157; t = 2.710, 2P <0.01).

A small number of patients (n = 4) were treated with gamma globulin injections. Mean maximum remissions were not significantly different (2P >0.05) before and after this treatment. Obviously, a greater number of patients must be investigated to evaluate its significance.

Possible causes of treatment failure

Plasma infusion was effective in some patients, a number of whom went into a long term remission. However in some other patients, mainly those with idiopathic GN, there was no clinical benefit from this treatment and the results have proved disappointing.

I shall discuss below the possible causes of plasma treatment failure:

1. Choosing the wrong patients:

In some of our patients who had glomerulonephritis and uraemia (such as K.G. and W.W.), it seems that the treatment was too late.

2. Choosing the wrong donor:

We selected the suitable plasma donors by an *in vitro* cross-reaction test (RIA), which is probably not a satisfactory enough method.

3. K-cell deficiency:

In patients with K-cell deficiency IgG therapy would be expected to fail. Therefore this seems to need other procedures rather than plasma infusion.

4. Inaccessible infection:

In this case the treatment is unable to reach and eliminate the infective agent due to some reason, e.g. poor blood supply as in infective endocarditis (Peters and Lachmann, 1974).

5. Inadequate amount of antibody supply:

In patient S.G. the second relapse was treated with intramuscular injection of 500 mg of gamma globulin. This amount induced complete

remission, whereas the third relapse was treated with 250 mg of gamma globulin and the remission was not complete. This fact seems unlikely to be true for other patients who were treated with an equal amount of plasma.

Conclusion

I would like briefly to summarise some of my thoughts on the patients' treatment which occupied much of my work.

It was obvious from the patient studies that plasma infusion induced a dramatic improvement in some patients, mainly in the group of patients with immune complex vasculitis which is due to SLE, PAN, MCTD, and some patients with pericarditis and erythema multiforme.

Plasma infusion is a benign therapy which, when successful, seems to have advantages over other drug treatment which are at present available and have obvious dangers, e.g. immunosuppressive agents and anticoagulants.

The important problem of transfusion of viral hepatitis through this treatment can be minimized by careful screening of the donors.

Some further suggestions have arisen during our work:

A - The short term remission which was induced after plasma infusion in the patient H.Z. may suggest that some patients need further transfusion every few weeks, which is time consuming. This leads one to suggest the establishment of a plasma bank for transfusion of such patients.

B - Blood transfusion in the patient FM with SLE induced complete remission for a long period of time. This may suggest that giving all blood components may be useful in some patients with immune complex disease.

C - Gamma globulin injection in some patients with pericarditis and erythema multiforme was very effective. This may suggest that giving gamma globulin alone may be useful and time saving in certain diseases.

These studies suggest that specific immunological means for the production of long or short term remission is possible in some patients with various immunological disorders ranging from immune complex disease, e.g. SLE, PAN, vasculitis, etc., to non-immune complex disease, e.g. pericarditis.

However to choose the right patient for this type of treatment we need further information and knowledge of the disease pathogenesis which is still obscure in the majority of immune complex diseases.

The results of plasma treatment which are part of the work in this thesis are obviously of a preliminary trial, designed to see if there were an effect worth further study. The results obtained point to a favourable effect, but there is a defect in this type of trial. It is that each patient is used as their own control, but at different stages of the disease. Thus the improved remission observed after treatment might be because the patient would have improved with time despite it. A controlled clinical trial of better design is clearly now required. In such a trial the patients would be divided into two groups, one of which was treated with plasma infusion or gamma globulin, and the other group of patients treated with a placebo, possibly their own plasma. Statistical analysis of the results of this trial should reveal any benefit of the plasma infusion.

In order to minimise the patient and investigator bias it would probably be necessary for the trial to be double blind with only a third person knowing from where the plasma came. The clinician in charge of the patient and the patients themselves would be unaware of the treatment until the end of the trial.

Thus the investigations in this thesis represent those of a pilot, feasibility, study of plasma infusion in various immune complex or infectious diseases. I hope that future investigations using longer follow-up and controlled placebo infusion will show more clearly the role of plasma treatment of these diseases.

REFERENCES

Abrass, C.K., Hall, C.L., Border, W.A., Brown, C.A., Glassock, R.J. & Coggins, C.H. (1980). Circulating immune complexes in adults with idiopathic nephrotic syndrome. Kidney Int., 17, 545.

Allison, A.C. (1979). Infectious diseases. In: Medical Immunology. 1st edition, ed. Irvine, W.J. pp.77-102. Edinburgh: Teviot Scientific Publications.

Allison, A.C., Houba, V., Hendrickse, R.G., de Petras, S., Edington, G.M. & Adeniyi, A. (1969). Immune complexes in the nephrotic syndrome of African children. Lancet, 1, 1232.

Alper, C.A., Colten, H.R., Rosen, F.S., Rabson, A.R., Macnab, G.M. & Gear, J.S.S. (1972). Homozygous deficiency of C3 on a patient with repeated infections. Lancet, 2, 1179.

Alpers, J.H., Steward, M.W. & Soothill, J.F. (1972). Differences in immune elimination in inbred mice: The role of low affinity antibody. Clin. Exp. Immunol., 12, 121.

Andres, G.A., Donadio, Jr., Elwood, C.M., McCluskey, R.T., Pirani, V.E., Pollak, V.E. & West, C.D. (1978). Glomerulonephritides. In: Immunologically Mediated Renal Disease, eds. McCluskey, R.T. & Andres, G.A. pp.11-69. New York: Marcel Dekker.

Anthony, B.A., Kaplan, E.L., Chapman, S.S., Quie, P.G. & Wannamaker, L.W. (1967). Epidemic acute nephritis with reappearance of type-49 streptococcus. Lancet, 2, 787.

Arieff, A.I. & Pinggera, W.F. (1972). Rapidly progressive glomerulonephritis treated with anticoagulants. Arch. Intern. Med. 129, 77.

Arnett, F.C. & Shulman, L.E. (1976). Studies in familial systemic lupus erythematosus. Medicine, 55, 313.

Atkins, R.C., Holdsworth, S.R., Glasgow, E.F. & Matthews, F.E. (1976). The macrophage in human rapidly progressive glomerulonephritis. Lancet, 1, 830.

Baldwin, D.S. (1977). Post streptococcal glomerulonephritis: A progressive disease? Am. J. Med. 62, 1.

Baldwin, D.S., Gluck, M.C., Lowenstein, J. & Gallo, G. (1977). Lupus nephritis: Clinical course as related to morphologic forms and their transitions. Am. J. Med., 62, 12.

Baldwin, D.S. & McCluskey, R.T. (1968). Renal involvement in systemic lupus erythematosus, periarteritis nodosa, scleroderma and cryoglobulinemia. In: Structural Basis of Renal Disease, ed. Becker, E.L. pp.428-461. New York: Hoeber Medical Division.

Ballard, H.S., Eisinger, R.P. & Gallo, G. (1970). Renal manifestations of Henoch-Schönlein syndrome in adults. Am. J. Med., 49, 328.

Barratt, J. & Naish, P. (1979). A simple radiolabelled rheumatoid factor binding assay for the measurement of circulating immune complexes. J. Immunol.Methods, 25, 137.

Bayer, A.S., Theofilopoulos, A.N., Eisenberg, R.A., Dixon, F.J. & Guze, L.B. (1976). Circulating immune complexes in infective endocarditis. New Engl. J. Med. 295, 1500.

Beaucher, W.N., Garman, R.H. & Condemi, J.J. (1977). Familial lupus erythematosus: Antibodies to DNA in household dogs. New Engl. J. Med. 296, 982.

Beirne, J.G. & Brennan, J.T. (1972). Glomerulonephritis associated with hydrocarbon solvents. Mediated by antiglomerular basement membrane antibody. Arch. Environ. Health, 25, 365.

Bellanti, J.A. (ed.)(1978) Immunology I I. Philadelphia: W.B.-Saunders.

Benacerraf, B., Portter, J.L., McCluskey, R.T. & Miller, F. (1960). The pathologic effects of intravenously administered soluble antigen-antibody complexes. J. Exp. Med., 111, 195.

Bendinelli, M., Ruschi, A., Campa, M. & Toniolo, A. (1975). Depression of humoral and cell-mediated immune responses by Coxsackiviruses in mice. Experienta, 31, 1227.

Benoit, F.L., Rulon, D.B., Theil, G.B., Doolan, P.D. & Watten, R.H. (1964) Goodpasture's syndrome: A clinicopathologic entity. Am. J. Med., 37, 424.

Berger, J. (1969). IgA glomerular deposits in renal disease. Transplant. Proc. 1, 939.

Berlyne, G.M. (1979). Renal involvement in the collagen diseases. In: Renal Disease, 4th edition, eds. Black, Sir D. & Jones, N.F. pp.653-686. Oxford: Blackwell Scientific Publications.

Berthoux, F.C., Gagne, A., Sabatier, J.C., Ducret, F., Le Petit, J.C., Marcellin, M., Mercier, B. & Brizard, C.P. (1978). HLA-BW35 and mesangial IgA glomerulonephritis. New Engl. J. Med., 298, 1034.

Black, D.A.K. (1970). Diagnosis in renal disease. Brit. Med. J., 2, 315.

Black, J.A., Challacombe, D.N. & Ockenden, B.G. (1965). Nephrotic syndrome associated with bacteraemia after shunt operations for hydrocephalus. Lancet, 2, 921.

Block, S.R. & Christian, C.L. (1975). The pathogenesis of systemic lupus erythematosus. Am. J. Med., 59, 453.

Boulton-Jones, J.M., Sissons, J.G.P., Evans, D.J. & Peters, D.K. (1974) Renal lesions of subacute infective endocarditis. Brit. Med. J., 2, 11.

Braunstein, G.D., Lewis, E.J., Galvanek, E.G., Hamilton, A. & Bell, W.R. (1970). The nephrotic syndrome associated with secondary syphilis: An immune deposit disease. Am. J. Med., 48, 643.

Brettle, R., Peters, D.K. & Batchelor, J.R. (1978). Mesangial IgA glomerulonephritis and HLA antigen. New Engl. J. Med., 298, 200.

Brewerton, D.A., Caffrey, M., Hart, F.D., James, D.C.O., Nicholls, A. & Sturrock, R.D. (1973). Ankylosing spondylitis and HL-A27. Lancet, 1, 904.

Briggs, W.A., Johnson, J.P., Teichman, S., Yeager, H.C. & Wilson, C.B. (1979). Antiglomerular basement membrane antibody-mediated glomreulonephritis and Goodpasture's syndrome. Medicine, 58, 348.

Brown, D.L. (1975). Biological activities of complement. In: The Immune System: a course on the molecular and cellular basis of immunity, 2nd edition, eds. Hobart, M.J. & McConnel, I. pp.274-286. Oxford: Blackwell Scientific Publications.

Bruton, C.O.C. (1952). Agammaglobulinemia. Pediatrics, 9, 722.

Buckley, R.H. (1977). Replacement therapy in immunodeficiency. In: Recent Advances in Clinical Immunology, vol.1, ed. Thompson, R.A. pp.219-244. Edinburgh: Churchill Livingstone.

Burkholder, P.M. (1974). Atlas of Human Glomerular Pathology. Hagerstown: Harper and Row.

Burton-Kee, E.J., Lehner, T. & Mowbray, J.F. (1979). Antigens in circulating immune complexes of Behcet's syndrome. In: Behcet's Syndrome: Clinical and immunological features, eds. Lehner, T. & Barnes, C.G. pp.45-54. London: Academic Press.

Cameron, J.S. (1979). The natural history of glomerulonephritis. In: Renal Disease, 4th edition, eds. Black, Sir D. & Jones, N.F. pp.329-380. Oxford: Blackwell Scientific Publications.

Cameron, J.S. (1979). Pathogenesis and treatment of membranous nephropathy. Kidney Int., 15, 88.

Cameron, J.S., Glasgow, E.F., Ogg, C.S. & White, R.H.R. (1970). Membrano-proliferative glomerulonephritis and persistent hypocomplementaemia. Brit. Med. J. 4, 7.

Cameron, J.S., Turner, D.R., Ogg, C.S., Sharpstone, P. & Brown, C.B. (1974). The nephrotic syndrome in adults with minimal change glomerular lesions. Quart. J. Med., 43, 461.

Canfied, C.J., Davis, T.E. & Herman, R.H. (1973). Hemorrhagic pulmonary-renal syndrome. New Engl. J. Med. 268, 230.

Carter, P.M. (1973). Immune complex disease. Ann. Rheum. Dis. 32, 265.

Chalopin, J.M., Rifle, G., Turc, J.M., Cortet, P. & Severac, M. (1980). Immunological findings during successful treatment of HBs-Ag associated polyarteritis nodosa by plasmapheresis alone. Brit. Med. J., 280, 368.

Churg, J., Habib, R. & White, R.H.R. (1970). Pathology of the nephrotic syndrome in children. Lancet, 1, 1299.

Colley, D.G. (1977). The immunopathology of schistosomiasis. In: Recent Advances in Clinical Immunology, No.1, ed. Thompson, R.A. pp.101-123. Edinburgh: Churchill Livingstone.

Colten, H.R., Levey, R.H., Rosen, F.S. & Alper, C.A. (1973). Decreased synthesis of C3 in membranoproliferative glomerulonephritis. J. Clin. Invest., 52, 20a.

Counaham, R., Winterborn, M.H., White, R.H.R., Heaton, J.M., Meadow, S.R., Bluett, N.H., Swetschin, H., Cameron, J.S. & Chantler, C. (1977). Prognosis of Henoch-Schonlein nephritis in children. Brit. Med. J., 2, 11.

Crofton, J. & Truelove, L. (1948). Apparent acute glomerulonephritis without albuminuria. Lancet, 2, 54.

Cypes, R.H. (1978). Mechanisms of immunity to parasitic diseases. In: Immunology II, ed. Bellanti, J.A. pp.418-436. Philadelphia: WB-Saunders.

Dambuyant, C., Burton-Kee, J., Mowbray, J.F. (1979). Demonstration of two disease specific antigens in circulating immune complexes. Clin. Exp. Immunol., 37, 424.

Darmady, E.M., Griffiths, W.J., Spencer, H., Mattingly, D., Stranak, F. & de Wardener, H.E. (1955). Renal tubular failure associated with polyarteritis nodosa. Lancet, 1, 378.

Davies, D.R., Tighe, J.R., Jones, N.F. & Brown, G.W. (1973). Recurrent haematuria and mesangial IgA deposition. J. Clin. Path., 26, 672.

Davis, A.E., Schneeberger, E.E., Grupe, W.E. & McCluskey, R.T. (1978). Membranoproliferative glomerulonephritis (MPGN type 1) and dense deposit disease (DDD) in children. Clin. Nephrol., 9, 184.

Day, N.K., Geiger, H., McLean, R., Michael, A. & Good, R.A. (1973). C2 deficiency: Development of lupus erythematosus. J. Clin. Invest., 52, 1601.

Day, N.K., Geiger, H., Stroud, R., de Bracco, B., Mancada, D., Windhorst, M.D. & Good, R.A. (1972). C1r deficiency: An inborn associated with cutaneous and renal disease. J. Clin. Invest. 51, 1102.

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.

de Wardener, H.E. (1973). The Kidney: An outline of normal and abnormal structure and function, 4th edition, Edinburgh: Churchill Livingstone.

Digeon, M., Laver, M., Riza, J. & Bach, J.F. (1977). Detection of circulating immune complexes in human sera by simplified assays with polyethylene glycol. J. Immunol. Methods, 16(2), 165.

Dixon, F.J. (1971). Experimental serum sickness. In: Immunological Diseases, vol. 1, 2nd edition, eds. Talmage, D.W., Rose, B., Sherman, W.B. & Vaughan, J.H. pp.253-264. Boston: Little, Brown.

Dixon, F.J., Feldman, J.D. & Vazquez, J. (1961). Experimental glomerulonephritis: The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. J. Exp. Med., 113, 899.

Dixon, F.J., Oldstone, M.B.A. & Tonietti, G. (1969). Virus-induced immune-complex-type glomerulonephritis. Transplant Proc., 1, 945.

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, 660.

Droz, D., Zanetti, M., Noel, L-H. & Leibowitch, J. (1977). Dense deposit disease. Nephron, 19, 1.

Drummond, K.N. & Andres, G.A. (1978). Glomerular disease lacking impressive evidence of immunological mechanisms. In: Immunology Mediated Renal Diseases, eds. McCluskey, R.T. & Andres, G.A. pp. 72-81. N.Y.: Marcel Dekker.

Drutz, D.J. & Mills, J. (1980). Immunity and infection. In: Basic and Clinical Immunology, 3rd edition, eds. Fudenberg, H.H., Sites, D.P., Caldwell, J.L. & Wells, J.V. pp.251-273. Los Altos: Lange Medical Publications.

Duffy, J.L. (1969). Myxovirus-like particles in lipoid nephrosis. New Engl. J. Med., 281, 562.

Duffy, J.L., Cinque, T., Grishman, E. & Churg, J. (1970). Intraglomerular fibrin, platelet aggregation, and subendothelial deposits in lipoid nephrosis. J. Clin. Invest., 49, 251.

East, J., Holborow, E.J. & Jaquet, H. (1967). Autoimmune reactions and virus-like particles in germ-free NZB mice. Lancet, 1, 755.

Edgington, T.S., Glassock, R.J. & Dixon, F.J. (1968). Autologous immune complex nephritis induced with renal tubular antigen. J. Exp.Med., 127, 555.

Edgington, T.S., Glassock, R.J. & Dixon, F.J. (1967). Autologous immune-complex pathogenesis of experimental allergic glomerulonephritis. Science, 155, 1432.

Edington, G.M. (1967). Pathology of malaria in West Africa. Brit. Med.J., 1, 715.

Evans, A.S., Rothfield, N.F. & Niederman, J.C. (1971). Raised antibodies titres to E.B. virus in systemic lupus erythematosus. Lancet, 1, 167.

Evans, D.J. (1974). Pathogenesis of membranous glomerulonephritis. Lancet, 1, 1143.

Eyres, K., Mallick, N.P. & Taylor, G. (1976). Evidence for cell-mediated immunity to renal antigens in minimal-change nephrotic syndrome. Lancet, 1, 1158.

Falls, W.F., Jr., Ford, K.L., Ashworth, C.T. & Carter, N.W. (1965). The nephrotic syndrome in secondary syphilis: Report for a case with renal biopsy findings. Ann. Intern. Med., 63, 1047.

Faulk, W.P. & Greenwood, B.M. (1977). Infectious diseases. In: Immunology in Medicine: A comprehensive guide to clinical immunology. eds. Holborow, E.J. & Reeves, W.G. pp.433-472. London: Academic Press.

Fawcett, W., Hilton, P.J., Jones, N.F. & Wing, A.J. (1971). Nephrotic syndrome in the elderly. Brit. Med. J., 2, 387.

Feldman, J.D. (1958). Election microscopy of serum sickness nephritis. J. Exp. Med., 108, 957.

Feldman, J.D., Mardiney, M.R. & Shuler, S.E. (1966). Immunology and morphology of acute post-streptococcal glomerulonephritis. Lab. Invest. 15, 283.

Fenner, F.J. & White, D.O. (1976). Medical Virology, 2nd edition. New York; Academic Press.

Filastre, J.P., Morel-Maroger, L. & Richet, G. (1971). Schönlein-Henoch purpura in adults. Lancet, 1, 1243.

Friend, P., Repine, J.E., Kin, Y., Clawson, C.C. & Michael, A.F. (1975). Deficiency of the second component of complement (C2) with chronic vasculitis. Ann. Intern. Med., 83, 813.

Gärtner, H.V., Fischbach, H., Wehner, H., Bohle, A., Edel, H.H., Kluthe, R., Scheler, F. & Schmülling, R.M. (1974). Comparison of clinical and morphological features of peri-(epi-extra) membranous glomerulonephritis. Nephron, 13, 288.

Gelfand, M.C., Frank, M.M. & Green, I. (1975). A receptor for the third component of complement in the human renal glomerulus. J. Exp. Med., 142, 1029.

Gelfand, M.C., Shin, M.L., Nagle, R.B., Green, I. & Frank, M.M. (1976). The glomerular complement receptor in immunologically mediated renal glomerular injury. New Engl. J. Med., 295, 10.

Gerber, M.A. & Paronetto, F. (1971). IgE in glomeruli of patients with nephrotic syndrome. Lancet, 1, 1097.

Germuth, F.G. (1953). A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. J. Exp. Med., 97, 257.

Germuth, F.G. & Rodriguez, E. (1973). Immunopathology of the Renal Glomerulus. Boston: Little, Brown.

Giangiacomo, J., Clearly, T., Cole, B.R., Hoffsten, P. & Robson, A.M. (1975). Serum immunoglobulins in the nephrotic syndrome. New Engl. J. Med., 293, 8.

Gilles, H.M. & Hendrickse, R.G. (1963). Nephrosis in Nigerian children: Role of plasmodium malariae, and effect of antimalarial treatment. Brit. Med. J. 2, 27.

Glassock, J.R. (1979). Clinical features of immunologic glomerular disease. In: Immunologic Mechanisms of Renal Disease. eds. Wilson, C.B., Brenner, M.B. & Stein, J.H. pp.255-322. New York: Churchill Livingstone.

Gluck, M.C., Gallo, G., Lowenstein, J. & Baldwin, D.S. (1973) Membranous glomerulonephritis: Evolution of clinical and pathologic features. Ann. Intern. Med. 78, 1.

Goke, D.J., Hsu, K., Morgan, C., Bombardieri, S., Lockshin, M. & Christian, C.L. (1970). Association between polyarteritis and Australia antigen. Lancet, 2, 1149.

Goldsmith, H.J., Cowan, M.A. & Gooder, E. (1958). Familial outbreak of acute glomerulonephritis due to Griffith type-1 streptococcus. Lancet, 2, 674.

Goodman, H.C., Lambert, P.H. & Mauel, J. (1979). Immunology of parasitic diseases. In: Principles of Immunology, 2nd edition, eds. Rose, N.R., Milgrom, F. & van Oss, C.J. pp.265-276. New York: Macmillan Publishing.

Goodman, J.W. & Wang, A.C. (1980). Immunoglobulins: Structure, diversity, and genetics. In: Basic and Clinical Immunology, 3rd edition, eds. Fudenberg, H.H., Sites, D.P., Caldwell, J.L. & Wells, J.V. pp.28-43. Los Altos: Lange Medical Publications.

Gutman, R.A., Striker, G.E., Gilliland, B.C. & Cutler, R.E. (1972). The immune complex glomerulonephritis of bacterial endocarditis. Medicine, 51, 1.

Gyorkey, F., Min, K.W., Sincovics, J.G. & Gyorkey, P. (1969). Systemic lupus erythematosus and myxovirus. New Engl. J. Med., 280, 333.

Habib, R., Kleinknecht, C., Gubler, M.C. & Levy, M. (1973). Idiopathic membranoproliferative glomerulonephritis in children: Report of 105 cases. Clin. Nephrol., 1, 194.

Harrington, J.C., Fenton, J.W. & Pert, J.H. (1971). Polymer-induced precipitation of antigen-antibody complexes: 'Precipiplex' reactions. Immunochemistry, 8, 413.

Hawn, C.V.Z. & Janeway, C.A. (1947). Histological and serological sequence in experimental hypersensitivity. J. Exp. Med., 85, 571.

Hayward, A.R. (1977). Immunodeficiency, No.6. London: Edward Arnold.

Heptinstall, R.H. (1974). Pathology of the kidney, 2nd edition. Boston: Little, Brown.

Herbert, W.J. & Wilkinson, P.C. (eds)(1977). A Dictionary of Immunology, 2nd Edition. Oxford: Blackwell Scientific Publications.

Herscowitz, H.B. (1978). Immunophysiology: Cell function and cellular interactions. In: Immunology II, ed. Bellanti, J.A. pp.151-202. Philadelphia: W.B. Saunders.

Heymann, W., Hackel, D.B., Harwood, S., Wilson, S.G.F. & Hunter, J.L.P.(1959). Production of nephrotic in rats by Freund's adjuvants and rat kidney suspensions. Proc. Soc. Exp. Biol. Med.N.Y., 100, 660.

Heyneman, D. & Welch, T.M. (1980). Parasitic diseases. In: Basic and Clinical Immunology, 3rd edition, eds. Fudenberg, H.H., Sites, D.P., Caldwell, J.L. & Wells, J.V. pp.677-690. Los Altos: Lange Medical Publications.

Hotchin, J. & Collins, D.N. (1964). Glomerulonephritis and late onset disease of mice following neonatal virus infection. Nature, 203, 1357.

Hughes, G.R.V. (1971). Significance of anti-D.N.A. antibodies in systemic lupus erythematosus. Lancet, 2, 861.

Hughes, G.R.V. (1975). Systemic lupus erythematosus. In: An Introduction to Clinical Rheumatology, 2nd edition, eds. Mason, M. & Currey, H.L.F. pp.132-155. London: Pitman Medical.

Hunter, W.M. (1973). Radioimmunoassay. In: Handbook of Experimental Immunology, ed. Weir, D.M. pp.17.1-17.35. Oxford: Blackwell Scientific Publications.

Hyman, L.R. & Burkholder, P.M. (1973). Focal sclerosing glomerulonephropathy with segmental hyalinosis: A clinical analysis. Lab. Invest., 28, 533.

Hyman, L.R. & Burkholder, P.M. (1974). Focal sclerosing glomerulonephropathy with hyalinosis: A clinical and pathologic analysis of the disease in children. J. Pediat., 84, 217.

Irvine, W.J. (ed.)(1979). Basic immunology. In: Medical Immunology, 1st edition. pp.3-37. Edinburgh: Teviot Scientific Publications.

James, J.A. (1972). Renal Disease in Childhood, 2nd edition. Saint Louis: C.V. Mosby.

Janeway, C.A. & Rosen, F.S. (1966). The gamma globulins: IV. Therapeutic uses of gamma globulin. New Engl. J. Med., 275, 826.

Jawetz, E., Melnick, J.L. & Adelberg, E.A. (1978). Review of Medical Microbiology, 13th edition. Los Altos: Lange Medical Publications.

Jenis, E.H., Teichman, S., Briggs, W.A., Sandler, P., Hollerman, C.E., Calcagno, P.L., Knieser, M.R., Jensen, G.E. & Valeski, J.E. (1974). Focal segmental glomerulosclerosis. Am. J. Med., 57, 695.

Jones, J.V., Cumming, R.H., Bucknall, R.C., Asplin, C.M., Fraser, I.D., Bothamley, J., Davis, P. & Hamblin, T.J.(1976). Plasmapheresis in the management of acute systemic lupus erythematosus? Lancet, 1, 709.

Julian, D.G. & Matthews, M.B. (1977). Diseases of the cardiovascular system. In: Davidson's Principles and Practice of Medicine, 12th edition, ed. MacLeod, J. pp.171-247. Edinburgh: Churchill Livingstone.

Kabat, E.A. (1963). Uses of hyperimmune human gamma globulin. New Engl. J. Med., 269, 247.

Kabat, E.A. & Mayer, M.M. (1961). Experimental Immunochemistry, 2nd edition. Springfield: Thomas.

Kaplan, E.L., Anthony, B.F., Chapman, S.S. & Wanamber, L.W. (1970). Epidemic acute glomerular nephritis associated with type 49 streptococcal pyoderma. Am. J. Med. 48, 9.

Kapoor, A., Mowbray, J.F., Porter, K.A. & Peart, W.S. (1980). Significance of haematuria in hypertensive patients. Lancet, 1, 231.

Kassirer, J.P. & Schwartz, W.B. (1961). Acute glomerularnephritis. New Engl. J. Med. 265, 686.

Kibwkamusake, J.W., Hutt, M.S.R. & Wilks, N.E. (1967). The nephrotic syndrome in Uganda and its association with quartan malaria. Quart. J. Med., 36, 393.

Kilbourne, E.D. & Loge, J.P. (1948). Comparative effects of continuous and intermittent penicillin therapy on formation of anti-streptolysin in hemolytic streptococcal pharyngitis. J. Clin. Invest. 27, 418.

Kobayashi, O., Wada, H., Okawa, K. & Tokeyama, I. (1977). Schönlein-Henoch's syndrome in children. In: Contributions to Nephrology, 4, ed. Ueda, Y. pp.48-71. Basel: Karger, S.

Kohler, P.F. (1973). Clinical immune complex disease: Manifestations in systemic lupus erythematosus and hepatitis B virus infection. Medicine, 52, 419.

Kushner, D.S., Armstrong, S.H., Dubin, A., Szanto, D.B., Markovitz, A., Madaros, B.P., Levine, J.M., River, G.L., Gynn, T.N. & Pendras J.P. (1961). Acute glomerulonephritis in the adult: Longitudinal, clinical, functional and morphological studies of rates of healing and progress of chronicity. Medicine (Baltimore), 40, 203.

Lachmann, P.J. (1975). The immunochemistry of complement. In: The Immune System: a course on the molecular and cellular basis of immunity. 2nd edition, eds. Hobart, M.J. & McConnell, I. pp.56-75. Oxford: Blackwell Scientific Publications.

Leaf, A. & Cotran, R.S. (1980). Renal Pathophysiology, 2nd edition. New York. Oxford: Oxford University Press.

Leibowitch, J., Leveille, M., Halwachs, L. & Wattels, S. (1980). Glomerulonephritides and hypocomplementemia: Pathophysiology and pathogenic implications. In: Advances in Nephrology, vol. 9, eds. Hamburger, J., Crosnier, J., Grünffld, J.P. & Maxwell, M.H. pp.295-314. Chicago: Year Book Medical Publishers.

Levinsky, R.J., Cameron, J.S. & Soothill, J.F. (1977). Serum immune complexes and disease activity in lupus nephritis. Lancet, 1, 564.

Levinsky, R.J., Malleson, P.N., Barratt, T.M. & Soothill, J.F. (1978). Circulating immune complexes in steroid-responsive nephrotic syndrome. New Engl. J. Med., 298, 126.

Lewis, E.J., Kallen, R.J. & Rowe, D.S. (1973). Glomerular localisation of IgE in lipoid nephrosis. Lancet, 1, 1395.

Lockwood, C.M., Boulton-Jones, J.M., Lowenthal, R.M., Simpson, I.J., Peters, D.K. & Wilson, C.B. (1975). Recovery from Goodpasture's syndrome after immunosuppressive treatment and plasmapheresis. Brit. Med. J., 2, 252.

Lockwood, C.M., Rees, A.J., Pearson, T.A., Evans, D.J., Peters, D.K. & Wilkinson, C.B. (1976). Immunosuppression and plasma-exchange in the treatment of Goodpasture's syndrome. Lancet, 1, 711.

Low, T.L.K., Liu, Y-S.V. & Putnam, F.W. (1976). Structure, function, and evolutionary relationships of FC domains of human immunoglobulins A, G, M and E. Science, 191, 390.

Magil, A.B., Price, J.D.E., Bower, G., Rance, C.P., Huber, J. & Chase, W.H. (1979). Membranoproliferative glomerulonephritis type 1: Comparison of natural history in children and adults. Clin. Nephrol., 11, 239.

Mallick, N.P. (1977). The pathogenesis of minimal change nephropathy. Clin. Nephrol., 7, 87.

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

Mannik, M. & Arend, W.P. (1971). Fate of performed immune complexes in rabbits and rhesus monkeys. J. Exp. Med., 134, 19.

Marchalonis, J.J. (1975). Lymphocyte surface immunoglobulins: Molecular properties and function as receptors for antigen are discussed. Science, 190, 20.

McConnell, I. (1976). The cells of the immune system. In: Advanced Medicine, No.12, ed. Peters, D.K. pp.1-13. London: Pitman Medical.

McCrory, W.W., Flisher, D.S. & Sohn, W.B. (1959). Effects of early embulation on the course of nephritis in children. Pediatrics, 24, 295.

McGregor, I.A., Turner, M.W., Williams, K. & Hall, P. (1968). Soluble antigens in the blood of African patients with severe plasmodium falciparum malaria. Lancet, 1, 881.

MacLennan, I.C.M. & Harding, B. (1974). Non-T cytotoxicity in vitro. In: Progress in Immunology II, 3, eds. Brent, L. & Holborough, J. pp.347-350. North-Holland: Amsterdam.

Mellors, R.C., Toshikazu, S., Aoki, T., Huebner, R.J., Krawczynski, K. (1971). Wild-type gross leukemia virus and the pathogenesis of the glomerulonephritis of New Zealand mice. J. Exp. Med., 133, 113.

Mery, J.Ph., Kourilsky, O., Morel-Maroger, L. & Adam, C. (1978). Partial lipodystrophy and glomerulonephritis without complement activation. New Engl. J. Med., 298, 1034.

Michael, A.F., Westberg, N.G., Fish, A.J. & Vernier, R.L. (1971). Studies on chronic membranoproliferative glomerulonephritis with hypocomplementemia. J. Exp. Med., 134, 208.

Migone, L., Olivetti, G., Allergi, L. & Dall'Aglio, P. (1980). Mesangio proliferative glomerulonephritis. Clin. Nephrol., 13, 219.

Miller, M.E. (1973). Uses and abuses of plasma therapy in the patient with recurrent infections. J. Allergy Clin. Immunol., 51, 45.

Mohammed, I., Ansell, B.M., Holborow, E.J., Bryceson, A.D.M. (1970). Circulating immune complexes in subacute infective endocarditis and poststreptococcal glomerulonephritis. J. Clin. Pathol. 30, 308.

Moran, C.J., Parry, H.G., Mowbray, J., Richards, J.D.M. & Goldstone, A.H. (1977). Plasmapheresis in systemic lupus eruthematosus. Brit. Med. J. 1, 1573.

Morel-Maroger, L. (1976). Histopathological analysis of glomerular diseases. In: Advanced Medicine, No.12, ed. Peters, D.K. pp.325-346. London: Pitman Medical.

Morris, P.J. (1979). HLA and disease. In: Medical Immunology, 1st edition, ed. Irvine, W.J. pp.69-76. Edinburgh: Teviot Scientific Publications.

Mowbray, J.F. (1977). New principles in determination of complement components in large numbers of sera. Transactions of the Merz + Dade Exploratory Seminar "Dudingen, ed. Rosalki, S.B. pp.72-77. Vienna: Hans Huber.

Mowbray, J.F. & Burton, E.J. (1976). Clinical relevance of the determination of circulating immune complexes. In: 8th International Course on Transplantation and Clinical Immunology. pp.35-43.

Nagi, A.H., Alexander, F. & Lannigan, R. (1971). Light and electron microscopical studies of focal glomerular sclerosis. J. Clin. Pathol., 24, 846.

Newman, W.J., Tisher, C.C., McCoy, R.C., Gunnells, J.C., Krueger, R.P., Clapp, J.R. & Robinson, R.R. (1976). Focal glomerular sclerosis: Contrasting clinical patterns in children and adults. Medicine, 55, 67.

Nezlin, R.S. (1977). Structure and Biosynthesis of Antibodies. New York: Plenum Publishing Co.

Ngu, J.L., Barratt, T.M. & Soothill, J.F. (1970). Immunoconglutinin and complement: Changes in steroid sensitive relapsing nephrotic syndrome of children. Clin. Exp.Immunol., 6, 109.

Northway, J.D., McAdams, A.J., Forristal, J. & West, C.D. (1969). A "silent" phase of hypocomplementemic persistentnephritis detectable by reduced serum β1c-globulin levels. J. Pediat., 74, 28.

Notkins, A.L., Maher, S., Scheele, C. and Goffman, J. (1966). Infectious virus-antibody complex in the blood of chronically infected mice. J. Exp. Med., 123, 81.

Nydeger, U.E., Ackermann, L.M., Lambert, P.H. & Miescher, P.A. (1972). A simple automated method for complement estimation in a continuous flow system. J. Immunol., 109, 910.

Ogg, C.S., Cameron, J.S. & White, R.H.R. (1968). The C'3 component of complement (β1c-globulin) in patients with heavy proteinuria. Lancet, 2, 78.

Ooi, Y.M., Vallota, E.H. & West, C.D. (1976). Classical complement component pathway activation in membranoproliferative glomerulonephritis. Kidney Int., 9, 46.

Ooi, Y.M., Vallota, E.H. & West, C.D. (1977). Serum immune complexes in membranoproliferative and other glomerulonephritides. Kidney Int., 11, 275.

Panem, S., Ordonez, N.G., Katz, A.I., Spargo, B.H. & Kirstein, W.H. (1978). Viral immune complexes in systemic lupus erythematosus: Specificity of C-type viral complexes. Lab. Invest., 39, 413.

Panem, S., Ordonez, N.G., Kirstein, W.H., Katz, A.I. & Spargo, B.H. (1976). C-type virus expression in systemic lupus erythematosus. New Engl. J.Med., 295, 470.

Paul, W.E. & Benacerraf, B. (1973). Functional specificity of thymus-dependent lymphocytes: A relationship between the specificity of T lymphocytes and their functions is proposed. Science, 195, 1293.

Perez, G.O., Bjornsson, S., Ross, A.H., Amato, J. & Rothfield, N. (1974). A mini epidemic of Goodpasture's syndrome: Clinical and Immunological studies. Nephron, 13, 161.

Pertschuk, L.P., Vulentin, J.C., Sutton, A.L. & Velazquez, L.A. (1976). Demonstration of antigen and immune complex in glomerulonephritis due to staphylococcus aureus. A.J. Clin. Pathol,, 66, 1027.

Pertschuk, L.P., Woda, B.A., Vulentin, J.C., Brigati, D.J., Soriano, C.B. & Nicastri, A.D. (1976). Glomerulonephritis due to staphylococcus aureus antigen. A.J.C.P., 65, 301.

Peters, D.K. (1979). Nephritis. In: Medical Immunology, 1st edition, ed. Irvine, W.J. pp.207-232. Edinburgh: Teviot Scientific Publications.

Peters, D.K. & Lachman, P.J. (1974). Immunity deficiency in pathogenesis of glomerulonephritis. Lancet, 1, 58.

Peters, D.K., Williams, D.G., Charlesworth, J.A., Boulton-Jones, J.M., Sisson, J.G.P. & Evans, D.J. (1973). Mesangiocapillary nephritis, partial lipodystrophy, and hypocomplementaemia. Lancet, 2, 555.

Pierides, A.M., Malasit, P., Morley, A.R., Wilkinson, R., Uldall, P.R., Kerr, D.N.S. (1977). Idiopathic membranous nephropathy: Quart. J. Med., 46, 163.

Pinching, A.J. (1980). Plasma exchange in immunologically mediated disease. In: Recent Advances in Clinical Immunology, No.2, ed. Thompson, R.A. Edinburgh: Churchill Livingstone.

Pincus, T., Haberkern, R. & Christian, C.L. (1968). Experimental chronic glomerulitis. J. Exp. Med., 127, 819.

Poon-King, T., Mohammed, I., Cox, R., Potter, E.V., Simon, N.M., Siegel, A.C. & Earle, D.P. (1967). Recurrent epidemic nephritis in South Trinidad. New Engl. J. Med., 277, 728.

Porter, R.R. (1967). The structure of antibodies. Scientific Am., 217, 81.

Porter, R.R. (1973). Structural studies of immunoglobulins. Science, 180, 713.

Potter, E.V., Siegel, A.C., Simon, N.M., McAninch, J. & Earle, D.P. (1968). Streptococcal infections and epidemic acute glomerulonephritis in South Trinidad. Tropical Pediat., 72, 871.

Proskey, A.J., Weatherbee, L., Weasterling, R.E., Greene, J.A. & Weller, J.M. (1970). Goodpasture's syndrome: A report of five cases and review of the literature. Am. J. Med., 48, 112.

Pussell, B.A., Lockwood, C.M., Scott, D.M., Pinching, A.J. & Peters, D.K. (1978). Value of immune complex assays in diagnosis and management. Lancet, 2, 359.

Rager-Zisman, B. & Allison, A.C. (1973). Effects of immunosuppression on Coxsackie B-3 virus infection in mice, and passive protection by circulating antibody. J. Gen. Virol., 19, 339.

Rees, B. Jr. (1980). Skin and appendages. In: Current Medical Diagnosis and Treatment, 19th edition, eds. Krupp, M.A. & Chatton, M.J. pp.37-81. Los Altos: Lange Medical Publications.

Riha, I., Haskova, V., Kaslik, J., Maierova, M. & Stransky, J. (1979). The use of polyethyleneglycol for immune complex detection in human sera. Molec. Immunol., 16, 489.

Robins, D.E., Lodd, A.T. (1962). Acute syphilitic nephrosis: Case report and review of literature. Am. J. Med., 32, 817.

Rodriguez-Iturbe, B., Carr, R.I., Garcia, R., Rabideau, D., Rubio, L. & McIntosh, R.M. (1980). Circulating immune complexes and serum immunoglobulins in acute poststreptococcal glomerulonephritis. Clin. Nephrol., 13, 1.

Roitt, I. (1980). Essential Immunology, 4th edition. Oxford: Blackwell Scientific Publications.

Row, P.G., Cameron, J.S., Turner, D.R., Evans, D.J., White, R.H.R., Ogg, C.S., Chantler, C., Brown, B. (1975). Membranous nephropathy. Long term follow-up and association with neoplasia. Quart. J. Med., 44, 207.

Roy, L.P., Fish, A.J., Vernier, R.I. & Michael, A.F. (1973). Recurrent macroscopic haematuria, focal nephritis, and mesangial deposition of immunoglobulin and complement. J. Pediat. 82, 767.

Ruddy, S., Carpenter, C.B., Chen, K.W., Knostman, J.N., Soter, N.A., Gotze, O., Muller-Eberhard, H.J. & Austen, K.F. (1975). Human complement metabolism: An analysis of 144 studies. Medicine, 54, 165.

Ruddy, S., Gili, I. & Austen, K.F. (1972). The complement system of man. New Engl. J. Med., 287, 489, 545, 642.

Ryan, G.B. & Majno, G. (1977). Acute inflammation. Am. J. Pathol., 86, 183.

Sato, M., Nakazora, H. & Ofiji, T. (1979). The pathogenetic role of staphylococcus aureus in primary human glomerulonephritis. Clin. Nephrol., 11, 190.

Schmidt, N.J., Lennatte, E.H. & Dennis, J. (1968). Characterization of antibodies produced in natural and experimental Coxsackie virus infections. J. Immunol., 100, 99.

Schwartz, W.B. & Kassirer, J.P. (1971). Clinical aspects of acute poststreptococcal glomerulonephritis. In: Diseases of the Kidney, ed. Strauss, M.P. & Welt, L.G. Boston: Little, Brown.

Seaton, A., Meland, J.M. & Lapp, N.L. (1971). Remission in Goodpasture's syndrome: Report of two patients treated by immunosuppression and review of literature. Thorax, 26, 683.

Shalhoub, R.J. (1974). Pathogenesis of lipoid nephrosis: A disorder of T-cell function. Lancet, 2, 556.

Shelly, W.B. (1967). Herpes simplex virus as a cause of erythema multiforme. J.A.M.A., 201, 153.

Silver, D.M., McKenzie, I.F.C. & Winn, H.J. (1972). Variations in the responses of C57 BL/10J and A/J mice to sheep red blood cells. J. Exp. Med., 136, 1063.

Sissons, J.G.P., West, R.J., Fallows, J., Williams, D.G., Boucher, B., Amos, N. & Peters, D.K. (1976). The complement abnormalities of lipodystrophy. New Engl. J. Med., 294, 461.

Sissons, J.G.P., Woodrom, D.F., Curtis, J.R., Evans, D.J., Gower, P.F., Sloper, J.C. & Peters, D.K. (1975). Isolated glomerulonephritis with mesangial IgA deposits. Brit. Med. J., 3, 611.

Sokolow, M. (1980). Heart and great vessels. In: Current Medical Diagnosis and Treatment, 19th edition, eds. Krupp, M.A. & Chatton, M.J. pp.161-260. Los Altos: Lange Medical Publications.

Soothill, J.F. & Steward, M.W. (1971). The immunopathological significance of the heterogeneity of antibody affinity. Clin. Exp. Immunol., 9, 193.

Spencer, H. & Rose, G.A. (1957). Polyarteritis nodosa. Quart. J. Med., 26, 43.

Spitzer, R.F., Vallota, E.H., Forristal, J., Sudora, E., Stitzel, A., Davis, N.C. & West, C.D. (1969). Serum C'3 lytic system in patients with glomerulonephritis. Science, 164, 436.

Sternlieb, I., Bennett, B. & Scheinberg, I.H. (1975). D-Penicillamine induced Goodpasture's syndrome in Wilson's disease. Ann. Inter. Med., 82, 673.

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

Stilmant, M.M., Bolton, W.K., Sturgill, B.C., Schmiti, G.W. & Couser, W.G. (1979). Crescentic glomerulonephritis without immune deposits: Clinico-pathologic features. Kidney Int., 15, 184.

Sturgill, B.C. & Worzniak, M.J. (1970). Stimulation of proliferation of 19S antibody-forming cells in the spleens of immunized Guinea-pigs after exchange transfusion. Nature, 228, 1304.

Taranta, A. & Uhr, J.W. (1971). Poststreptococcal diseases. In: Immunological Diseases, vol. 1, 2nd edition, eds. Talmage, D.W., Rose, B., Sherman, W.B. & Vaughan, J.H. pp.601-617. Boston: Little, Brown.

Tighe, J.R. (1975). Diseases of the kidney. In: Recent Advances in Pathology, No.2, eds. Harrison, C.V. & Weinberg, K. pp.131-154. Edinburgh: Churchill Livingstone.

Thompson, R.A. (1972). IgG3 levels in patients with chronic membrano-proliferative glomerulonephritis. Brit. Med. J., 1, 282.

Thompson, R.A. (1978). The Practice of Clinical Immunology, 2nd edition. London: Edward Arnold.

Thompson, R.A. & White, R.H.R. (1973). Partial lipodystrophy and hypo-complementaemic nephritis. Lancet, 2, 679.

Thomson, P.D., Barratt, T.M., Stokes, C.R., Turner, M.W. & Soothill, J.F. (1976). HLA antibodies and atopic features in steroid-responsive nephrotic syndrome of childhood. Lancet, 2, 765.

Trepo, C.G., Zucherman, 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 manifestations in polyarteritis nodosa. J. Clin. Pathol., 27, 863.

Treser, G., Semmar, M., Ty, A., Sagel, I., Franklin, M.A. & Lange, K. (1970). Partial characterization of antigenic streptococcal plasma membrane component in acute glomerulonephritis. J. Clin. Invest., 49, 762.

Tu, W.H., Shearn, M.A. & Lee, J.C. (1969). Acute diffuse glomerulonephritis in acute staphylococcal endocarditis. Ann. Inter. Med., 71, 335.

Turk, J.L. (1978). Immunology in Clinical Medicine, 3rd edition. London: Heinemann, W.

Turner, M.W. (1977). The immunoglobulins. In: Immunology in Medicine: A comprehensive guide to clinical immunology, eds. Holborow, E.J. & Reeves, W.G. pp.83-129. London: Academic Press.

Ueda, Y., Sakai, O., Yamagata, M., Kitajima, T. & Kawamura, K. (1977). IgA glomerulonephritis in Japan. In: Contributions to Nephrology, 4, ed. Ueda, Y. pp.36-47. Basel: Karger, S.

Unanue, E.R. & Dixon, F.J. (1965). Experimental glomerulonephritis: VI. The autologous phase of nephrotoxic serum nephritis. J. Exp. Med., 121, 175.

Unanue, E.R. & Dixon, F.J. (1967). Experimental glomerulonephritis: Immunological events and pathogenetic mechanisms. Advances Immunol., 6, 1.

Vallota, E.H., Forristal, J., Spitzer, R.E., Davis, N.I. & West, C.D. (1971). Continuing C3 breakdown after bilateral nephrectomy in patients with membranoproliferative glomerulonephritis. J. Clin. Invest., 50, 552.

Vallota, E.H., Götze, O., Spiegelberg, H.L., Forristal, J., West, C.D. & Müller-Eberhard, H.J. (1974). A serum factor in chronic hypocomplementemic nephritis distinct from immunoglobulins and activating the alternate pathway of complement. J. Exp. Med., 139, 1249.

Van de Putte, L.B.A., Brutel de la Rivierer, G. & van Breda Vriesman, P.J.C. (1974). Recurrent or persistent hematuria, sign of the mesangial immune-complex deposition. New Engl. J. Med., 290, 1165.

van der Peet, J., Arisz, L., Brentjens, J.R.H., Manink, J. & Hoedemaeker, Ph.J. (1977). The clinical course of IgA nephropathy in adults. Clin. Nephrol., 8, 335.

van Oss, C.J. (1979). The immunoglobulin. In: Principles of Immunology, 2nd edition, eds. Rose, N.R., Milgrom, F. & van Oss, C.J. pp.41-64. New York: Macmillan Publishing.

van Oss, C.J. & Grossberg, A.L. (1979). Antigen-antibody interactions. In: Principles of Immunology, 2nd edition, eds. Rose, N.R., Milgrom, F. & van Oss, C.J. pp.65-79. New York: Macmillan Publishing.

Voller, A., Draper, C.C., Tin Shwe & Hutt, M.S.R. (1971). Nephrotic syndrome in monkey infected with human quartan malaria. Brit. Med. J., 4, 208.

Ward, P.A. & Kibukamusoke, J.W. (1969). Evidence for soluble immune complexes in the pathogenesis of the glomerulonephritis in quartan malaria. Lancet, 1, 283.

Warthin, A.S. (1922). The excretion of spirochaete pallida through the kidneys. J. Infect. Dis., 30, 569.

Webster, R.G. & Laver, W.G. (1967). Preparation and properties of antibody directed specifically against the neuraminidase of influenza virus. J. Immunol., 99, 49.

West, C.D., McAdams, A.J., McConville, J.M., Davis, N.C. & Holland, N.H. (1965). Hypocomplementemic and normocomplementemic persistent (chronic) glomerulonephritis: Clinical and pathologic characteristics. J. Pediat., 67, 1089.

Westberg, N.G., Naff, G.B., Boyer, J.J. & Michael, A.F. (1971). Gomerular deposition of properdin in acute chronic glomerulonephritis with hypo-complementemia. J. Clin. Invest,, 50, 642.

White, R.H.R., Glasgow, E.F. & Mills, R.J. (1970). Clinicopathological study of the nephrotic syndrome in childhood. Lancet, 1, 1353.

Whitworth, J.A., Leibowitz, S., Kennedy, M.C., Cameron, J.S. & Chantler, C. (1976). IgA glomerular disease. Clin. Nephrol., 5, 33.

WHO Scientific Group (1976). The role of immune complexes in disease.

Williams, R.C. & Gibbons, R.J. (1972). Inhibition of bacterial adherence by secretory immunoglobulin A: A mechanism of antigen disposal. Science, 177, 697.

Wilmers, M.J., Cunliffe, A.C. & Williams, R.E.O (1954). Type-12 streptococci associated with acute haemorrhagic nephritis. Lancet, 2, 17.

Wilson, C.B. & Dixon, F.J. (1972). Immunological aspects of glomerulonephritis. In: Renal Disease, 3rd edition, ed. Black, Sir D. pp.275-294. Oxford: Blackwell Scientific Publications.

Wilson, C.B. & Smith, R.C. (1972). Goodpasture's syndrome associated with influenza A2 virus infection. Ann. Intern. Med., 76, 91.

Woodroffe, A.J., Border, W.A., Theofilopoulos, A.N., Gotze, O., Glassock, R.J., Dixon, F.J. & Wilson, C.B. (1977). Detection of circulating immun complexes in patients with flomerulonephritis. Kidney Int., 12, 268.

Yokoska, H., Nagase, M., Maeda, T. & Koide, K. (1978). Mesangial IgA glomerulonephritis clinicopathophysiological study of 85 cases. In: Contributions to Nephrology, 9, eds. Kobayashi, K., Maeda, Nagoya & Oshima, K. pp.101-110. Basel: Karger, S.

Young, C.R., Ebringer, A. & Archer, J.R. (1978). Immune response inversion after hyperimmunisation: Possible mechanism in the pathogenesis of HLA-linked diseases. Ann. Rheum. Dis., 37, 152.

Zabriskie, J.B. (1971). The role of streptococci in human glomreulonephritis. J. Exp. Med., 134, 180s.

Zech, P., Colon, S., Labeeuw, M., Bernheim, J. & Blanc-Brunat, N. (1978). Nephrotic syndrome in procainamide induced lupus nephritis. Clin. Nephrol. 11, 218.

Zubler, R.H., Perrin, L.H., Creighton, W.D. & Lambert, P.H. (1977). Use of polyethylene glycol (PEG) to concentrate immune complexes from serum or plasma samples. Ann. Rheum. Dis., 36, (suppl I), 23.

ADDITIONAL REFERENCES

Bartolotti, S.R. & Peters, D.K. (1978). Delayed removal of renal-bound antigen in decomplemented rabbits with acute serum sickness. Clin. Exp. Immunol., 32, 199.

Bartolotti, S.R. & Peters, D.K. (1979). Complement fixation in acute serum sickness : assembly of glomerular-bound C3 convertase. Clin. Exp. Immunol., 37, 391.

Czop, J. & Nussenzweig, V. (1976). Studies on the mechanism of solubilisation of immune precipitates by serum. J. Exp. Med., 143, 615.

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.

Jones, V. & Orlans, E. (1981). Isolation of immune complexes and characterisation of their constituent antigens and antibodies in some human disease : a review. J. Immunol. Methods, 44, 249.

Maini, R.N. (1979). Immune complexes in rheumatic diseases. IN: Advance Medicine, No. 15. Ed: Peter, S.H. & John, R.M. Pitman Medical, London. pp104-118.

Takahashi, M., Tack, B.F. & Nussenzweig, V. (1977). Requirements for the solubilisation of immune aggregates by complement. J. Exp. Med., 145, 86.

Theofilopoulos, A.N. & Dixon, F.J. (1979). The biology and detection of immune complexes. Advances in Immunology, 28, 89.