Immun

MEDICAL GRAND ROUNDS

University of Texas Health Science Center at Dallas
Southwestern Medical School

May 10, 1984

Hugo E. Jasin, M.D.

IMMUNE COMPLEX DISEASES IN PERSPECTIVE - PARADIGM LOST.

"No theory ever agrees with all the facts in its domain, yet it is not always the theory that is to blame. Facts are constituted by older ideologies, and a clash between facts and theories may be proof of progress. It is also a first step in our attempt to find the principles implicit in familiar observational notions."

Paul K. Feyerabend, "Against Method" (1)

INTRODUCTION

The development and evolution of our knowledge concerning the pathogenic role of circulating antigen-antibody complexes in vasculitis and glomerulonephritis has provided a theoretical framework of considerable explanatory power when applied to the multiple clinical manifestations of immune complex (IC) diseases in humans (2). However, in spite of its strong empirical underpinnings, this scientific construct appears to be no different than most other scientific paradigms (3). Once a paradigm has been established, scientists generate large amounts of empirical data from experiments predesigned to confirm the reigning paradigm. However, a trickle of observations that do not conform to the theory accumulate gradually until it becomes necessary to modify the original postulation to accommodate the available data. Finally, a new paradigm able to explain the old and new observations usually dethrones the old formulation. In recent years, a multitude of clinical and experimental observations have accumulated which cannot be fitted easily into the mold provided by a simple model of tissue injury mediated by circulating IC. In this review, I will attempt to illustrate with clinical examples the difficulties in correlating observed clinical manifestations with the pathophysiologic mechanisms presupposed by the original theory.

Mechanisms of Tissue Injury

a. Deposition of immune complexes in vessel walls and tissues. As antigen-antibody complexes make their appearance in the circulation, they may be either taken up by the reticuloendothelial system and circulating phagocytic cells, or they may deposit in blood vessel walls and tissues, setting the stage for subsequent injury. There is abundant evidence from work in experimental animals that the process of IC deposition in vessel walls and tissues is not a passive one (4,5). Large soluble complexes capable of inducing tissue injury cannot penetrate vessel walls unless there is a local increase in vascular permeability. Thus, IC deposition in tissues can be induced by simultaneous infusion of agents that cause liberation of vasoactive amines (6-8). Subsequent studies have demonstrated several in vivo mechanisms where IC interacting with plasma proteins and circulating cells induce the liberation of histamine, serotonin and other mediators from platelets and basophils. Some of the mechanisms involved are depicted in Figure 1.

Recognition of the importance of local increases in vascular permeability for IC deposition has potential therapeutic importance. In the experimental animal, administration of antagonists of vasoactive amines results in a decrease in deposition of IC in chronic experimental glomerulonephritis (9,10). Administration of methysergide, a serotonin antagonist, early in life, decreased the severity of IC nephritis in NZB/W mice (11). However, the course of autologous IC nephritis in rats could not be altered by administration of methysergide even when used in combination with other anti-inflammatory and immunosuppressive agents (12). It is surprising that practically no data is available on the importance of this mechanisms in human IC disease. Kniker employed

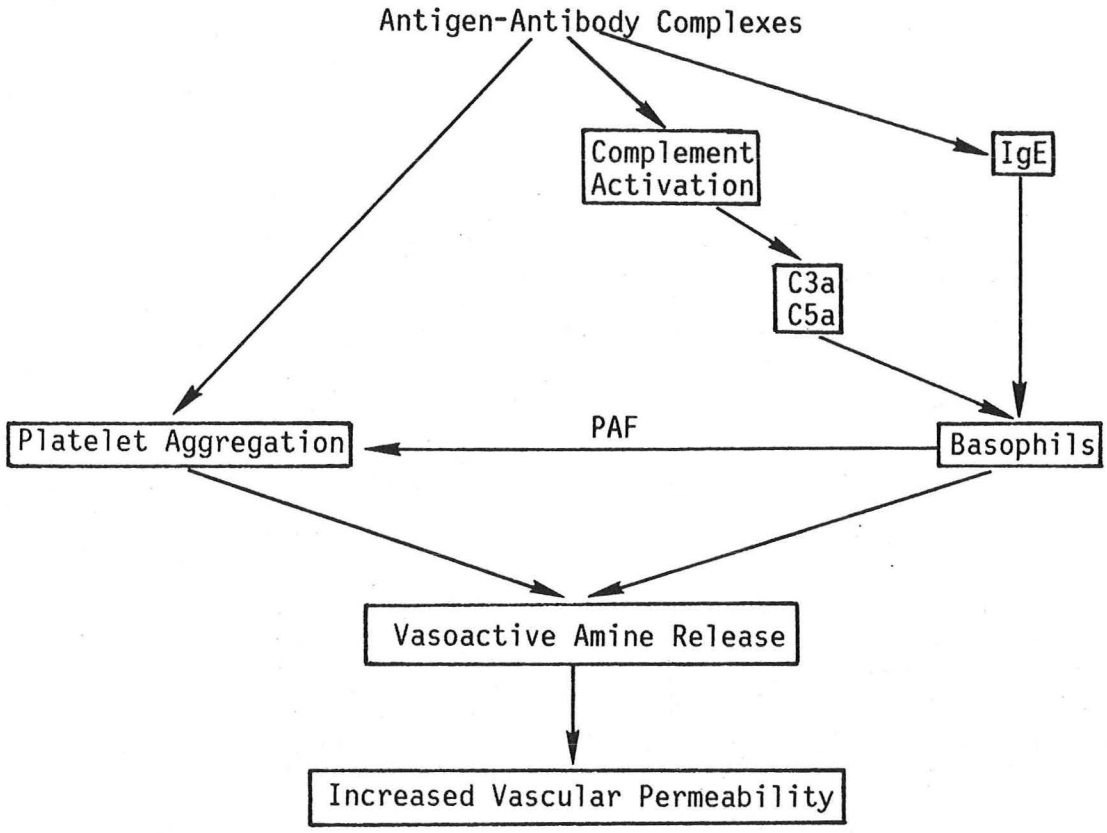
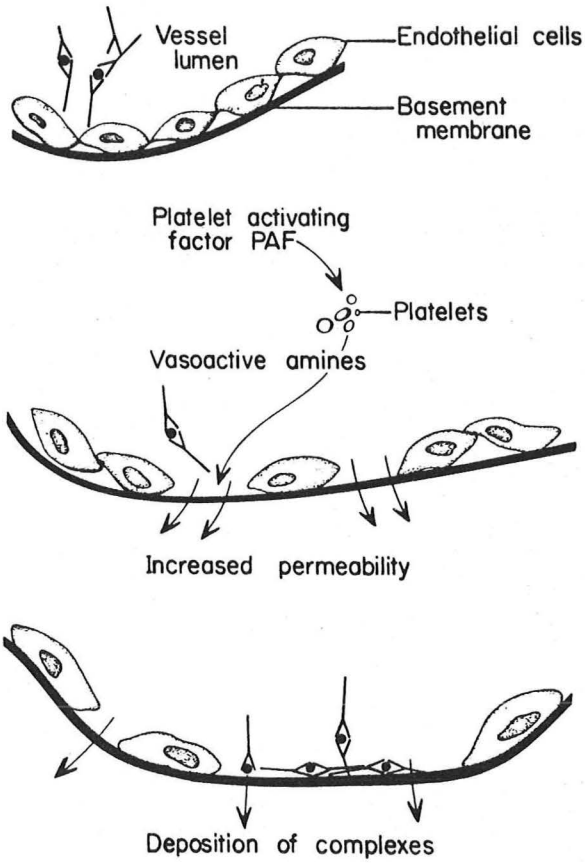


Figure 1. Mechanisms of vasoactive amine release by immune complexes

antagonists of vasoactive amines prospectively in a group of patients treated preventatively with diphtheria antitoxin. The incidence of symptoms of serum sickness was less than 5% in the treated group. This contrasted with 25% of untreated controls showing evidence of serum sickness (13).

Hydrodynamic forces are also important in tissue deposition of complexes. Arteritic lesions in experimental animals occur most commonly at bifurcations of large arteries and in artificially constricted areas of vessels (6). Moreover, the intensity of lesions in serum sickness is known to increase in the presence of hypertension (14).



From R.C. Williams, Jr., (2)

Figure 2. Mechanism of deposition of immune complexes in vessel walls.

b. Mechanisms of tissue damage. Once the IC are lodged in tissues, a series of events may result in pathologic changes leading to acute vasculitis characterized by massive infiltration of polymorphonuclear leukocytes. The human prototype of this acute process would correspond to the necrotizing arteritis of polyarteritis nodosa, the arteritis of serum sickness, and the vasculitis seen at the sites of Arthus reactions. In the kidneys, a similar picture is commonly present at the level of the glomerular capillaries in acute post-streptococcal glomerulonephritis. The IC deposited at the sites of injury induce local activation of the complement cascade with the consequent generation of powerful chemotactic factors derived from the 5th component of complement, C5. This process leads to the accumulation of neutrophiles and platelets which would contribute further to the changes in vascular permeability. In acute IC disease, the phagocytic cells are usually successful in disposing of the complexes in about 24 hours. For this

reason, evidence of IC deposition in areas of acute vasculitis may not be detectable in skin biopsies of lesions that are older than 1 or 2 days. However, during the process of phagocytosis and enzymatic digestion of complexes, the cells release large amounts of lysosomal enzymes which are able to attack and digest the structural elements of vessel walls such as basal membranes and internal elastic lamina. The importance of complement activation and the neutrophile in the mediation of this type of acute vasculitis is highlighted by experimental evidence showing that the lesions are completely inhibited if complement is depleted (15) or if neutrophiles are eliminated (16) when immune complexes enter the circulation.

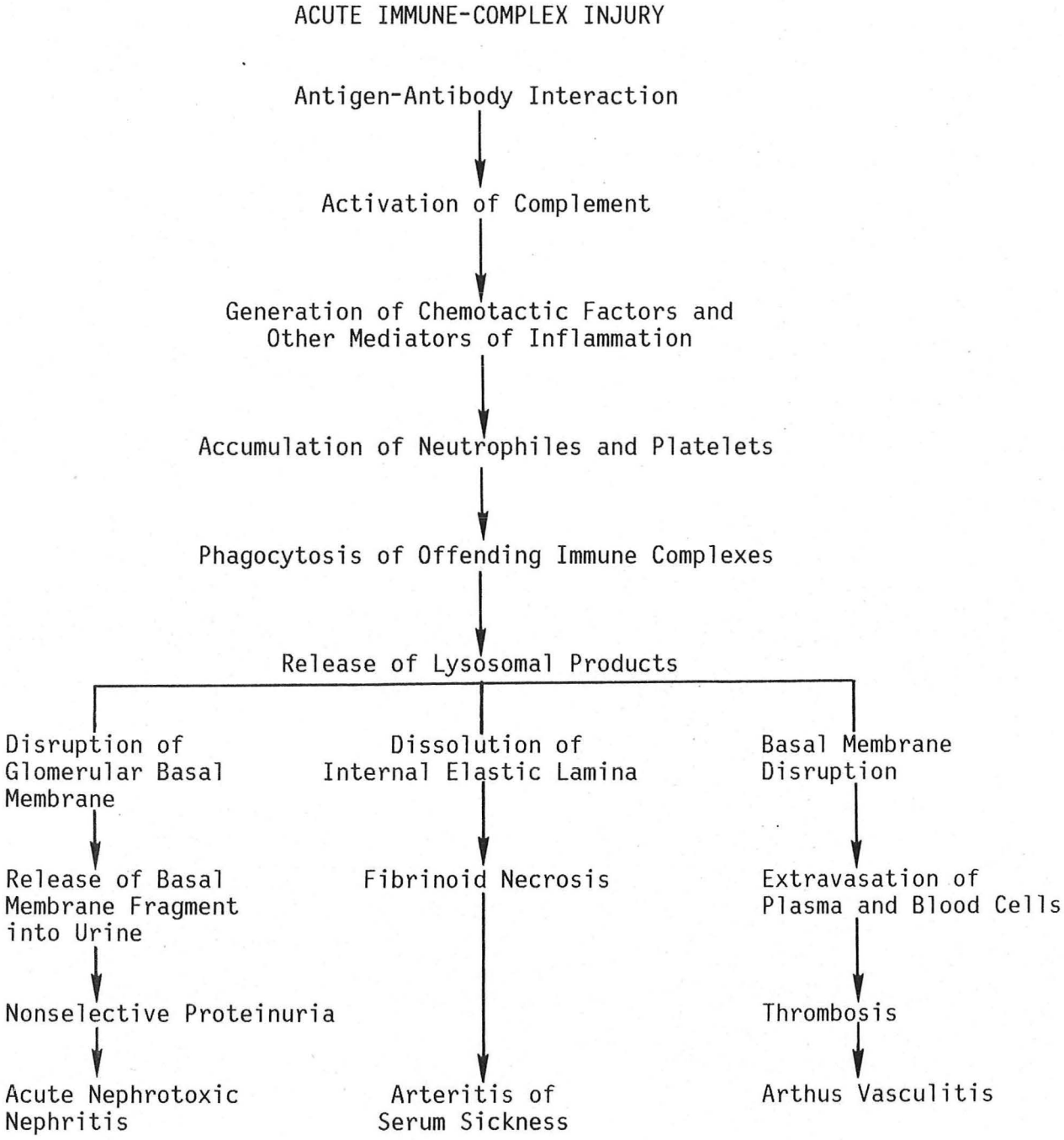


Figure 3. Acute injury mediated by immune complexes

The clinical patterns in patients with evidence of acute IC injury can have a wide range of severity depending on the many factors that modulate the expression of disease. Thus, the pathologic picture can range from an evanescent urticarial rash probably induced by release of vasoactive amines within the capillary lumen to massive necrosis of viscera and limbs following thrombosis of large vessels.

THE GAMUT OF VASCULAR WALL DAMAGE

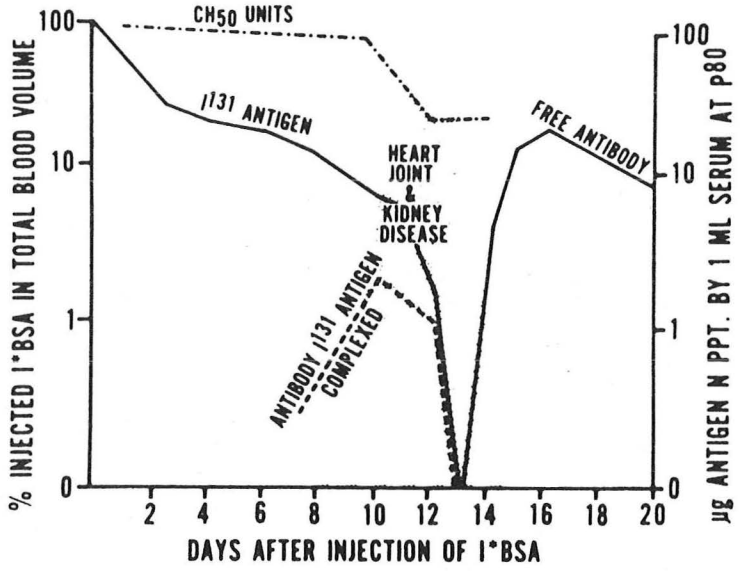
Vascular Change	Pathologic Manifestation	Gross Lesion
Increased permeability and endothelial swelling	Edema	Urticaria
More severe increase in permeability	Hemorrhage	Purpura-Petechia
Invasion of vessel wall by PMN Endothelial swelling - occlusion Fibrinoid necrosis	Hemorrhage	Palpable Purpura Bullae
Thrombosis	Ischemia Ischemic Necrosis	Necrotic ulcers Gangrene

Figure 4. Gross and microscopic changes indicative of vascular wall injury.

It should be emphasized that the pathophysiology described applies only to the acute vasculitides. In diseases where IC accumulate over a prolonged period of time, the pathologic picture, and presumably the mechanisms of tissue injury are different. In crescentic and chronic glomerulonephritis where the presence of IC is still prominent, accumulation of neutrophiles at the level of the glomeruli is uncommon. It is likely that in these diseases, the bone-marrow derived macrophage (17) may play an important role in tissue injury, at least in the crescentic glomerulonephritis group. In addition, recent work suggests that the activation of the terminal components of complement may also be important in the induction of proteinuria in membranous nephritis (18). The terminal complement components C6 to C9 are normally responsible for cell lysis. Activation of the complement cascade results in the assembly of membrane attack complex units which insert themselves in cell membranes creating hydrophillic channels that result in hemolysis, bacteriolysis, etc. In rabbits with congenital absence of C6, with consequent interruption of the sequence of activation, induction of experimental membranous glomerulonephritis is not associated with the early proteinuria seen in most animals with an intact complement cascade. Thus, it is likely that the same mechanism may play a role in the induction of glomerular basement membrane dysfunction in human membranous nephritis.

Experimental Acute Serum Sickness

Much of our knowledge concerning the pathophysiology of IC mediated injury derives from the animal models of acute and chronic IC disease. In rabbits given one large dose of a protein antigen, the development of arteritis and glomerulitis coincides with the detection of soluble IC in circulation, and an abrupt decrease in complement levels (5). Soon after the disappearance of circulating antigen and appearance of free antibody, the animals usually make a complete recovery of renal function. There are other aspects of this experimental model that illustrate the considerable variability of responses to the administration of large amounts of foreign antigen. In the human, as mentioned above, infusion of large amounts of horse serum proteins induces symptoms of serum sickness in no more than 20% of the population at risk. Similarly, in the rabbit model, no more than 30 per cent of the animals express some evidence of vasculitic lesions.



From Cochrane and Koffer (5)

Figure 5. Acute serum sickness model.

When the antibody response of the individual animals is examined closely, it is apparent that only the animals that produce moderate amounts of antibody show evidence of disease. A large proportion of rabbits produce antibody rapidly and in large amounts resulting in very rapid elimination of antigen and no evidence of long-lasting circulating complexes. A third group of animals does not mount an antibody response to the injected antigen. In either case, the rabbits do not develop vasculitis or renal disease. These considerations underline the importance of host factors and the biologic properties of the IC in modulating the expression of disease.

The Precipitin Curve

From the example described above, it is readily apparent that the quality and quantity of antigen and antibody able to generate IC in circulation are of paramount importance in the subsequent induction of tissue injury and clinical evidence of disease.

Analysis of the classical quantitative precipitin curve provides some clues of the importance of the relative amounts of antigen and antibody in the determination of the biologic properties of the resulting complexes.

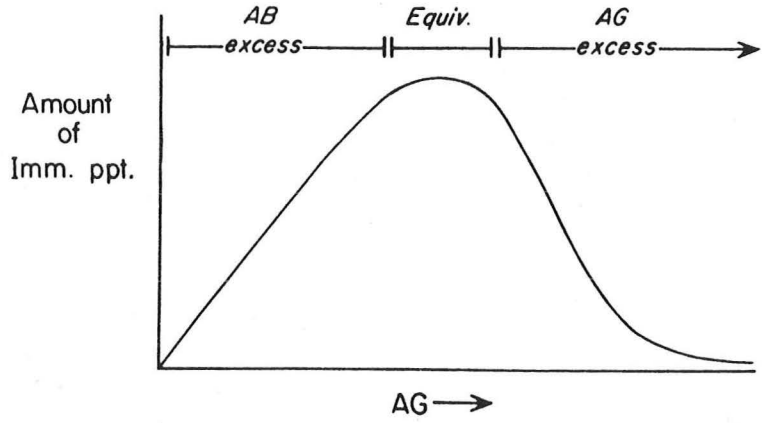
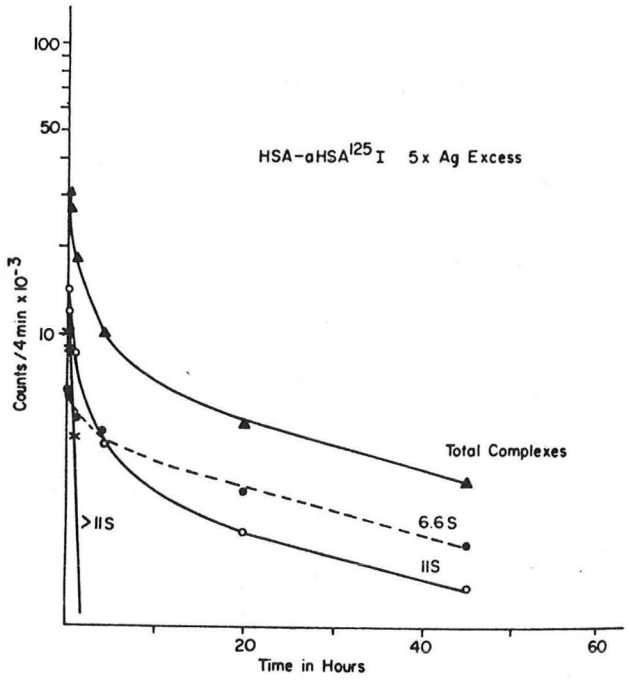


Figure 6. The quantitative precipitin reaction.

When the concentration of serum antibodies is in relative excess to the concentration of available antigen, the resulting complexes tend to be large and insoluble. In addition, they are usually able to activate and fix complement very efficiently. The same considerations apply to the complexes formed when optimal concentrations of antibody bind the available antigen. In this situation the circulating complexes have a very short half-life in circulation and little chance of being deposited in vessel-walls and glomeruli. However, as the relative amounts of antigen increase, the complexes become smaller and more soluble but they are still able to fix complement briskly. More importantly, as the size of the complexes diminish, the rate of disappearance from circulation also decreases, affording a greater opportunity for the smaller complexes to be trapped in tissues. An example of the large differences in clearance rates of complexes of different sizes is shown in Figure 7 (19). As the relative amounts of antigen increase to extreme antigen excess, the small complexes resulting do not fix complement and are not able to lodge in tissues in appreciable amounts (20).



From Mannick et al (19)

Figure 7. Disappearance curves of immune complexes of different sizes.

A striking clinical example of the importance of the relative concentrations of circulating antigen and antibody is described below.

Case Report. K.M. (PMH 35 36 61)

This 53 year-old white female had a long and complicated course covering 13 years and 1400 pages of medical records. A brief chronological summary follows:

> In 1958, the patient underwent total gastrectomy and splenectomy for lymphosarcoma. Following post-operative radiation, no evidence of recurrence was found.
>
> In 1969, the patient was admitted three times with gram-negative pneumonia. During her second admission, she was found to have leg ulcerations suggestive of vasculitis. Work-up revealed the presence of cryoglobulins with a cryocrit of 5%. The cryoglobulins were identified as being of the mixed type composed of IgM-rheumatoid

factor and IgG. Serum complement levels were normal (155 Units).

In 1970, she was admitted twice with lobar pneumonia which was successfully treated with antibiotics.

In May of 1970, serum complement levels dropped to 30 Units.

Her last admission in May of 1971 was prompted by the appearance of progressive dyspnea, orthopnea and pedal edema. Physical exam revealed revealed skin ulcerations on the right leg. There was dullness and moist rales in the left lung base. The heart was not enlarged. No murmurs or gallops were heard. There was 3+ pedal edema. The hospital course was marked by a rising BUN and creatinine and a falling hematocrit. A renal biopsy was compatible with proliferative glomerulonephritis. Terminally, the patient developed a LUL pneumonia and left-sided pneumothorax. She failed to respond to antibiotics and peritoneal dialysis. The patient expired on the 38th hospital day. Autopsy studies revealed disseminated malignant lymphoma, lymphocytic type, and necrotizing arteritis involving kidneys, adrenals, pancreas, colon, gall bladder and ovaries. The diagnosis of diffuse membranoproliferative glomerulonephritis was confirmed.

Comment. This patient had mixed IgM-IgG cryoglobulinemia for at least two years. Skin vasculitis was the only clinical manifestation of her disease. She developed hypogammaglobulinemia and diffuse glomerulonephritis in the last few months of her life. Figure 8 shows the remarkable sequence of events that probably precipitated the rapidly progressing renal disease and widespread necrotizing vasculitis.

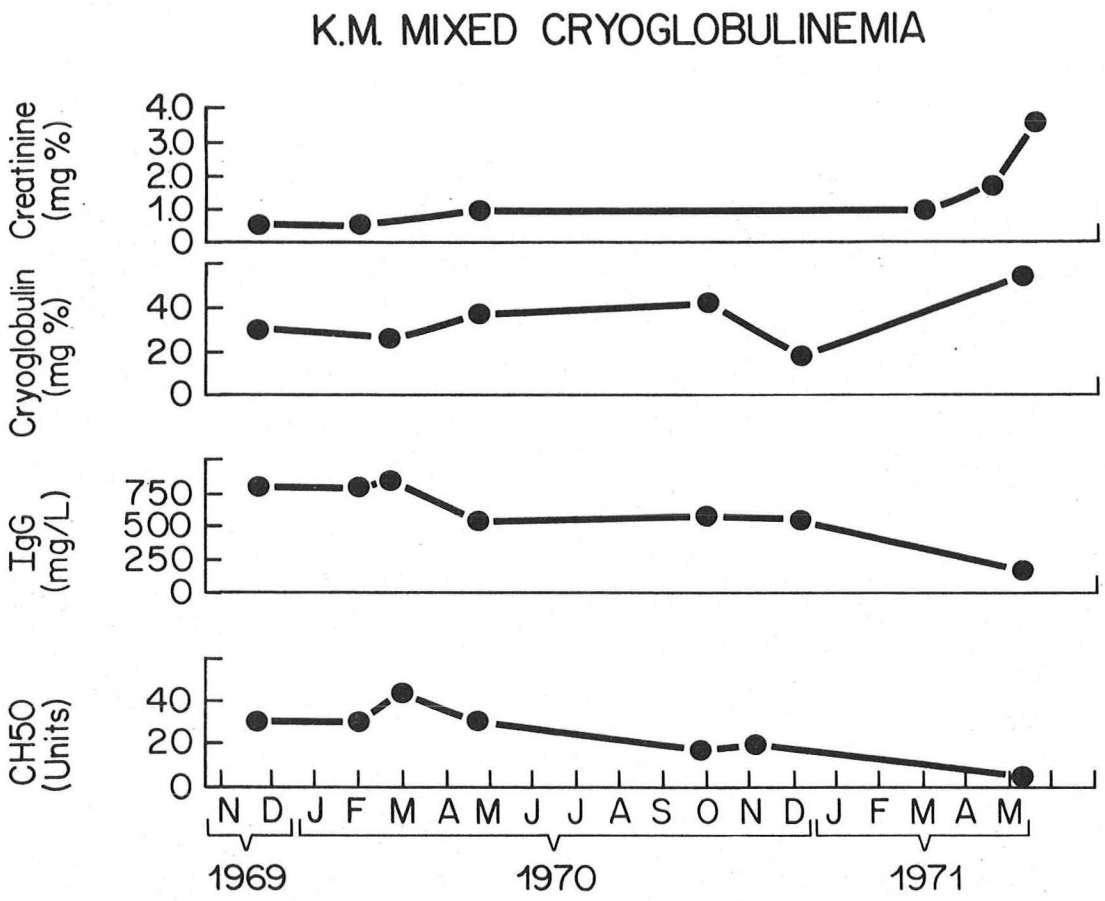


Figure 8. Clinical course of a patient with mixed cryoglobulinemia and hypogammaglobulinemia.

In 1969, the patient demonstrated only skin vasculitis with no evidence of visceral involvement. At this time her serum hemolytic complement levels were moderately low (30 units), and remained unchanged until September, 1970. Serum cryoglobulins ranged from 30 to 41 mg %. IgG concentrations demonstrated a significant drop to about 50 per cent of normal in March of 1970. During her last admission, the renal biopsy demonstrated severe membranoproliferative glomerulonephritis, coinciding with a precipitous drop of serum IgG to 150 mg/L, an increase in cryoglobulin concentration to 50 mg %, and a concomitant decrease in serum complement to very low levels (7.5 Units). Since her mixed cryoglobulin was composed of IgM-rheumatoid factor and its antigen IgG, it was felt that as the serum IgG (antigen) concentrations dropped, the resulting IgM-RF-IgG complexes became larger and more phlogogenic. This impression was confirmed by the simple experiment depicted in Table 1. Aliquots of purified cryoglobulin from this patient were added to cryoglobulin-free sera from 1969, and to a hypogammaglobulinemic specimen obtained during her last admission. It is readily apparent that twice as much insoluble cryoglobulin could be recovered from the

serum from the time that the patient had widespread necrotizing vasculitis and glomerulitis.

TABLE 1

Solubility of Cryoglobulins Added to Sera of Patient K.M. With Normal and Low IgG Concentrations

Date	IgG mg/L	Cryoglobulin Added mg	Cryoglobulin Recovered mg
12-09-69	780	1.0	0.2
05-29-71	150	1.0	0.4

This patient illustrates the importance of one of the many factors that may determine whether a given patient with circulating IC may develop clinical manifestations of disease.

The relative concentrations of reactant molecules is only one of the many characteristics of IC relevant to their pathogenicity. Table 2 lists a few of the better known factors that are probably important with regard to pathogenicity.

TABLE 2

Characteristics of Immune Complexes Relevant to Pathogenicity

Size
Valence of Antigen
Combining Ratios
Affinity
Immunoglobulin Class
Complement-fixing Capacity
Physicochemical Nature of the Antigen

Modified from Inman and Day (21)

It is also important to realize that host factors play a pivotal role in the physiologic handling of complexes generated as part of the process of elimination of foreign antigens. Thus, although circulating IC may be detectable in a large number of patients with infectious processes, only a few develop clinical manifestations of vasculitis or glomerulitis (2). It is likely that the outcome of any given event involving the invasion

of a foreign antigen, the ensuing immune response, and the generation of pathogenic IC depends on the interplay of a number of variables. Some of the host factors that determine the fate of immune complexes are listed in Table 3.

TABLE 3

Immune Complex Dynamics

Event	Variables
Encounter With Antigen	Single versus sustained exposure Route of Acquisition Nature of Antigen
Antibody Synthesis	Genetic Control of the Immune Response Prior Encounter with Antigen
Immune Complex Formation	In Situ versus Circulating
Fate of Immune Complexes Clearance by the RES	RES Capacity Modulation of Fc and C3b Receptors
Deposition in Tissues	Blood Flow Permeability Changes Antigen or Antibody Affinity for Tissues Anatomical Site Cellular Receptors Prior Tissue Damage Drug Effects

Modified from Inman and Day (21)

Time does not permit to discuss these factors in detail. However, a few clinical examples will serve to illustrate the importance of some of the characteristics listed in the table.

Importance of Anatomical Site of Deposition of Immune Complexes

The role of IC in the pathogenesis of the renal damage associated with systemic lupus erythematosus (SLE) is firmly established (22). An intriguing finding in this condition is the frequent presence of immunoglobulins and complement components deposited at the basement membrane area of the skin in a characteristic band-like pattern (23-25). It should be emphasized that whereas in discoid lupus, immunoglobulins are found exclusively in lesional skin, in SLE the lupus band test is

positive in uninvolved skin in over 50% of patients with active disease (25). Moreover, the group of patients with severe nephritis and presumably large amounts of complexes lodged in the glomerular capillary walls have the highest incidence of positive lupus-band tests in uninvolved skin (25). These observations underscore the importance of the anatomical location of the trapped complexes in the development of tissue injury.

TABLE 4

Relationship Between the Lupus Band Test and Renal Histology

Histologic Classification	Per Cent Positive LBT
PGN MGN	81
Mes. G. Normal	17

From Gillian et al. (25)

It should be pointed out that the specificities of the antibodies present both at the level of the glomerular capillary and epidermal basement membranes are probably very similar. In either case, the predominant autoantibodies eluted from tissues react with nuclear antigens, particularly DNA (24,26). The work of Dr. J. Gilliam has greatly contributed to elucidate the mechanism of deposition of IC under the epidermis (27). He found that the incidence of positive lupus-band tests is directly related to the turnover rate of epidermal cells and sun exposure in any area of the skin. It was also observed that immunoglobulin deposition occurred only in areas of keratinized epidermis suggesting that in situ cell death was a prerequisite. Finally, Dr. Gilliam demonstrated that DNA molecules had strong affinity for the epidermal basement membrane.

TABLE 5

Frequency of Subepidermal Immunoglobulin At Different Sites In SLE Patients

Biopsy Site	Per Cent Positive LBT
Flexor forearm	55
Extensor forearm	75
Deltoid	80

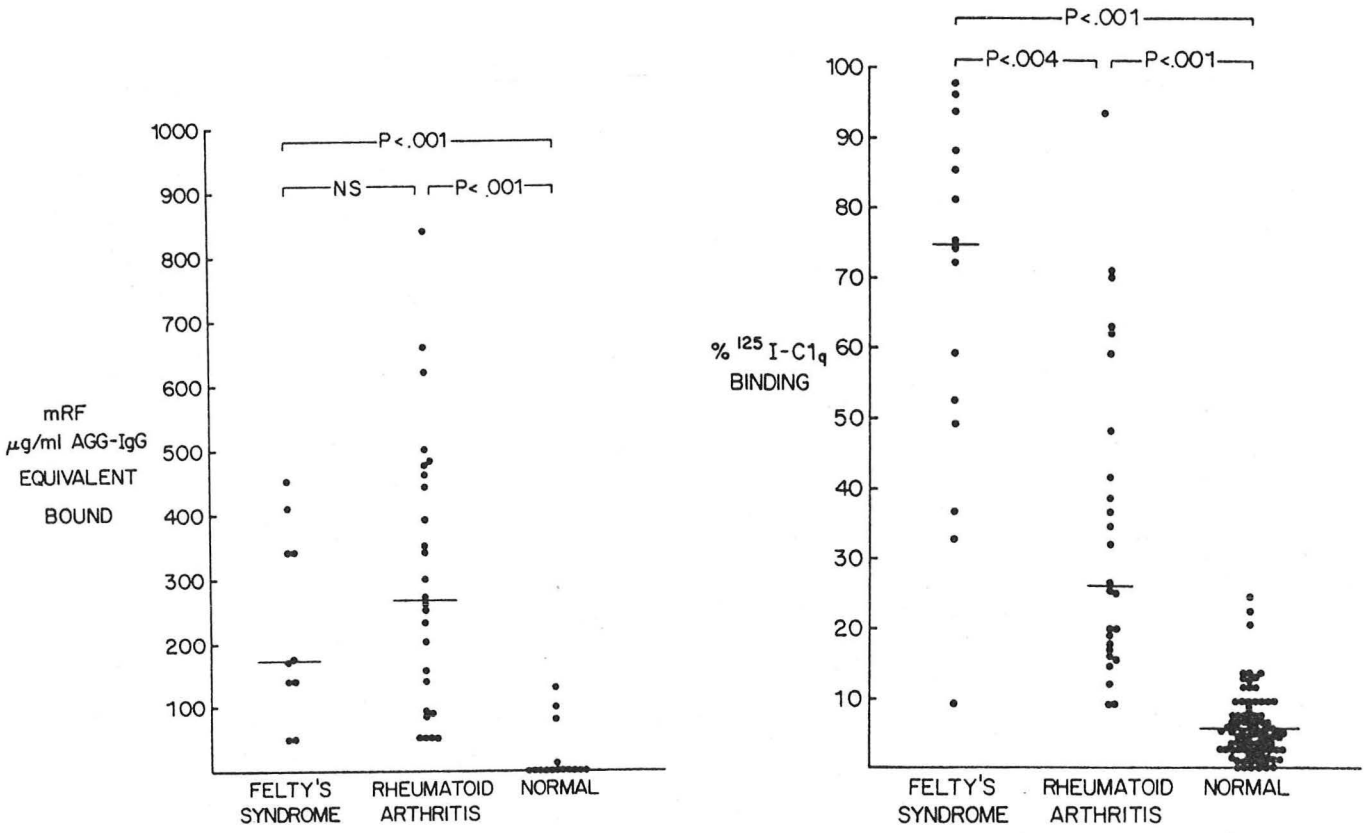
On the basis of these observations it was concluded that the immune deposits formed in situ as a result of the interaction of free DNA originating in dead cells diffusing from the epidermis, and anti-DNA antibodies normally present in the dermis of patients with SLE. There is one important unanswered question remaining: why are these complement-fixing immune deposits unable to initiate and maintain an inflammatory reaction? It is clear that other, as yet unknown factors must contribute to the development of a local inflammatory response.

Further illustration of the importance of the anatomical localization of IC is provided by the studies dealing with the pathogenesis of CNS involvement in patients with systemic lupus. CNS vasculitis, presumably IC-mediated, is unusual in SLE even though early clinical observations (28) showing an association of skin and visceral vasculitis with CNS involvement suggested a similar pathogenesis for both processes. Thus, the infrequent detection of vasculitic lesions on post-mortem examination of specimens from patients dying of diffuse CNS involvement has been puzzling. A possible solution for this apparent conundrum has been provided by two observations. Atkins et al (29) showed that immunoglobulin and complement deposits were frequently found in the choroid plexus of patients with SLE. However, these deposits were found in patients regardless of whether they had CNS involvement or not. The last piece of the puzzle was provided by recent observations on antineuronal antibodies in patients with SLE. The titers of brain-reactive antibodies in SLE sera tend to be higher in patients with CNS manifestations (30,31). More compelling evidence for their role in the pathogenesis of CNS disease has been reported by Bluestein et al (32). These authors have shown that the CSF of almost 80% of patients with CNS lupus contained high titers of anti-neuronal antibodies. These were found in only 10 per cent of lupus patients without CNS involvement. These findings suggest that IC may localize in CNS vascular structures, disrupting the blood brain barrier, increasing vascular permeability, and allowing the brain reactive autoantibodies to gain access to the CNS. This example illustrates that IC may also have a more subtle, indirect role in the mediation of disease.

Importance of Complement-Fixing Abilities of Immune Complexes

In our discussion of the pathogenic mechanisms involved in the mediation of tissue injury by IC emphasized the importance of complement activation. Thus, the complement fixing ability of complexes associated with any given clinical entity may determine the extent and severity of the clinical manifestations. Felty's syndrome is a good example of the role of complement fixing IC in the modulation of disease expression. Felty's syndrome is characterized by the triad of rheumatoid arthritis, splenomegaly and neutropenia. Although the mechanism of neutropenia is not completely understood, previous studies by Dr. Eric Hurd in our unit (33) suggested that the neutropenia in Felty's syndrome may be related to ingestion of IC by the circulating polymorphonuclear leukocytes. It is also of interest that ingestion of IC by the neutrophiles render these cells defective with regard to chemotaxis (34) and bacterial killing (35). Such cells are prone to increased margination (36) and splenic sequestration (37). Thus, ingestion of IC by polymorphonuclear

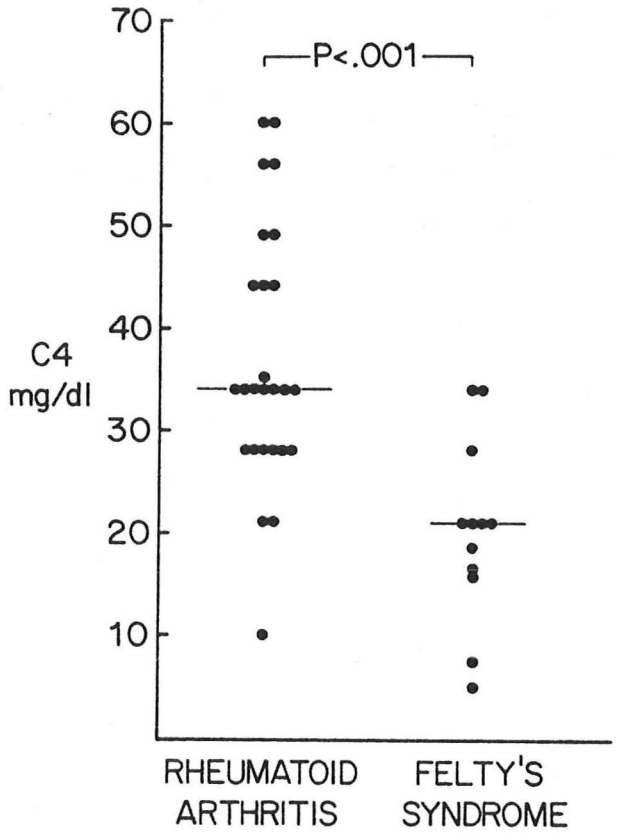
leukocytes may be responsible for the development of hypersplenism and increased susceptibility to infections in these patients. For these reasons, it was of interest to compare the nature of circulating IC in patients with uncomplicated rheumatoid arthritis and in a group of patients with Felty's syndrome (38). We employed two methods for the detection of IC: the monoclonal rheumatoid factor technique, and the Clq binding method to be described later. The former is used to measure all types of IC, whereas the Clq binding method detects complement-fixing IC exclusively.



From Hurd et al. (38)

Figure 9. Immune complexes in Felty's syndrome, uncomplicated rheumatoid arthritis and normal sera measured by the monoclonal rheumatoid factor (left) and ^{125}I-Clq binding techniques (right).

The results in Figure 9 show that there was little difference in the tenor of total IC between Felty's and uncomplicated rheumatoid arthritis. However, the concentration of complement-binding IC measured by the ^{125}I-Clq assay was significantly higher in the group of patients with Felty's syndrome. Moreover, as shown in Figure 10, the levels of the 4th component of complement in the sera of Felty's patients were lower than the controls, suggesting that increased in vivo complement consumption was taking place in the patients with Felty's syndrome.



From Hurd et al. (38)

Figure 10. Serum C4 levels in rheumatoid arthritis and Felty's syndrome.

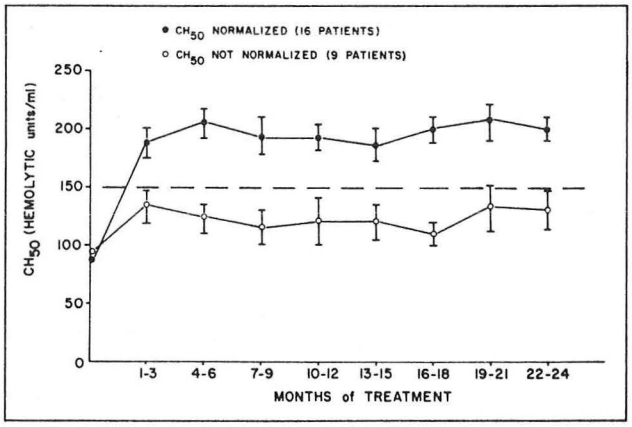
A further example of the importance of complement-fixing IC is provided by the large group of patients with SLE and nephritis. There is compelling evidence that glomerular deposition of IC play a major role in the development of the more severe form of renal involvement in SLE, i.e., the diffuse proliferative glomerulonephritis.

TABLE 6

Composition of Immune Complexes Eluted From Kidneys Obtained From SLE Patients

1) Anti-native DNA
2) Anti-single-strand DNA
3) Anti-nucleoprotein
4) Anti-ribonucleoprotein
5) Anti-Sm
6) Anti-Ro

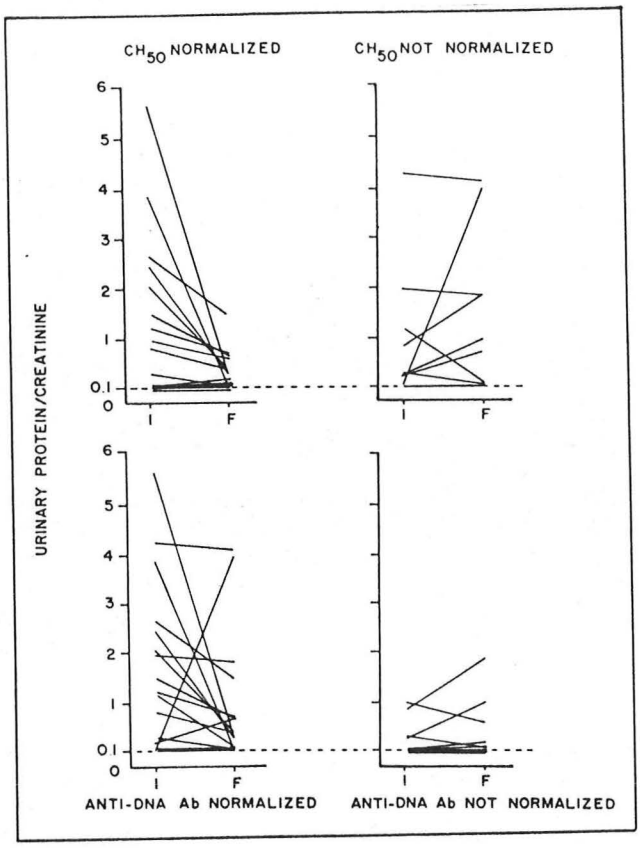
Moreover, the classic studies of Koffler et al (26) have implicated the autoantibodies directed against DNA as the major antigen-antibody system found in the glomeruli of patients with diffuse renal involvement. The IC extracted from diseased kidneys are significantly enriched with respect to anti-DNA antibody concentration compared to the serum of the same patients. In addition, immunoglobulin, complement components, and DNA itself are found by immunofluorescence staining in the majority of kidney biopsies obtained from patients with active disease. Electron microscopy studies have localized the IC to the subendothelial side of the glomerular capillary, in accordance with the hypothesis that these complexes originate in the circulation and eventually lodge between the endothelial cells and the glomerular basal membrane. The evidence for the importance of circulating IC in the generation of tissue injury in diffuse proliferative glomerulonephritis is considerably strengthened by the complementing data obtained from clinical studies correlating lupus renal disease and serologic findings. One of the main points supporting such evidence is the very good relationship existing between decreased serum complement levels and active nephritis (39-42).



From Appel et al. (42)

Figure 11. Serum levels of total hemolytic complement in 25 patients with SLE.

Appel et al. (42) carried out a prospective study of 25 patients with SLE on the effect of normalization of serum complement on the course of lupus nephritis. In 16 of 25 patients complement levels were maintained within the normal range for two years. Urinary protein excretion decreased or remained low in all 16. Repeat renal biopsies performed in 10 of these 16 patients disclosed stabilization or diminution of glomerular disease. In the 9 patients in whom complement levels could not be normalized in spite of aggressive treatment, urinary protein excretion stayed high or increased. Five of six repeat renal biopsies showed worsening of glomerular disease. At the end of five years of follow up, there was still a trend toward stabilization of renal histology, creatinine clearance, serum creatinine, and a lower mean dose of corticosteroids in the group with normalized complement levels.



From Appel et al. (42)

Figure 12. Corrected urinary protein excretion in the groups of patients with normalized and low complement levels.

TABLE 7

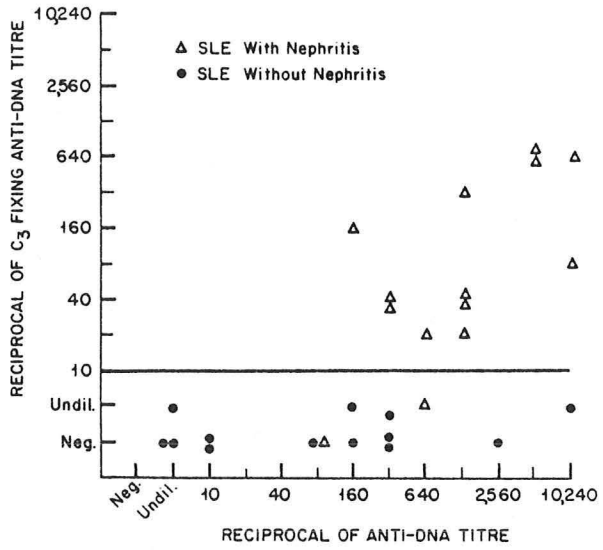
Assessment of the Histologic Change of the Initial Versus the Final Renal Biopsy Specimen in 19 Patients with SLE Grouped by Normalization of CH50

Serial Biopsies	CH50 Controlled	CH50 Uncontrolled
Improved	3	0
Unchanged	5	4
Slightly Worse	4	1
Moderately→severely worse	0	2

From Jarrett et al. (40)

Evidence for the direct correlation between complement-fixing anti-DNA antibodies in serum and severe renal disease has also been

obtained by several groups (43-45). Using an indirect immunofluorescence technique, Drs. Sontheimer and Gilliam (45) have shown an excellent correlation between the presence of complement-fixing anti-native DNA antibodies and active renal disease in SLE.



From Sontheimer and Gilliam (45)

Figure 13. Relationship between complement-fixing and non-complement-fixing anti-DNA antibodies in SLE with and without nephritis.

In their work, they showed that 12 of 14 patients with active lupus nephritis exhibited significant titers of complement fixing anti-DNA in their serum, whereas none of 13 patients without nephritis had titers equal to or greater than 1:10.

In Situ Formation Vs Deposition of Immune Complexes

The good clinical correlations discussed above between circulating IC, complement consumption, and lupus diffuse proliferative glomerulonephritis apply only to this histologic type of renal disease. The evidence for deposition of circulating IC in membranous glomerulonephritis is lacking. In this histopathologic type, the IC are localized mainly to the epithelial side of the glomerular basal membrane. In the non-SLE membranous nephritis, the subepithelial IC appear in the virtual absence of their circulating counterparts (46,47). Similarly, in lupus membranous nephritis, evidence for intravascular complement consumption and for the presence of circulating complexes of the size that may be able to move across the basal membrane is missing (48). Thus, it is likely that in this type of renal disease, IC may well form in situ (49) by mechanisms similar to those described earlier for the formation of subepidermal immune deposits. Moreover, several well studied properties of the renal glomerular structures are believed to facilitate anchoring and in situ growth of IC.

TABLE 8

Factors Facilitating Formation of Subepithelial Immune Complexes

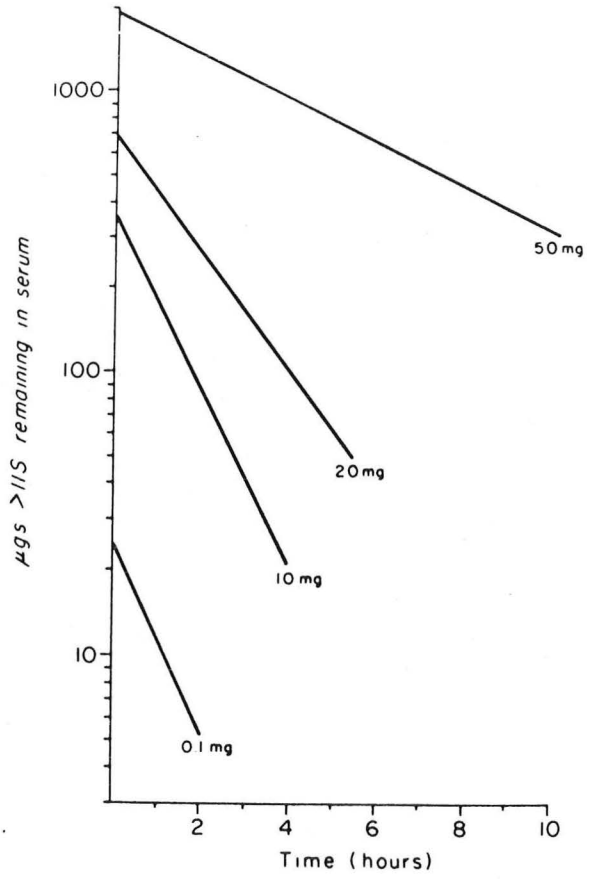
		References
1)	Interaction between antigens and glomerular basal membrane	50,51
2)	Interaction between antibody and glomerular basal membrane	52,53
3)	Presence of complement receptors	54,55
4)	Antiglomerular basal membrane antibodies	56,57

In SLE, the affinity of DNA molecules for collagen and basal membranes may provide the nucleus for the in situ growth of DNA-anti DNA IC (50). A similar type of interaction has been shown to occur between cationic antigens or immunoglobulins, and free anionic charges in the basal membrane. Infusion of cationic antigen (51) or cationic IC (52) results in early formation of subepithelial deposits in the experimental animal.

In situ formation of IC does not exclude complement activation from the pathogenesis of membranous nephropathy. It is likely that C3b receptors present in the epithelial cell membrane may well contribute to the retention of complement fixing IC. In addition, there is evidence that complement activation may mediate the development of immunological glomerular basement membrane injury as described earlier (18).

Removal of Circulating Immune Complexes From Circulation

Deposition of IC at the tissue level is determined not only by the rate of formation but also by the rate of clearance from the circulation mediated by the reticuloendothelial system (RES) (58-60). The RES has a finite capacity for the clearance of circulating IC. Thus, the half-life of these IC is in part determined by their concentration in blood. The saturability of this system has been clearly demonstrated by elegant studies in experimental animals (59). Figure 14 compares the clearance rates of different amounts of IC infused in mice. It is clear that the rate of disappearance of soluble complexes decreases as the amounts infused increase (59).

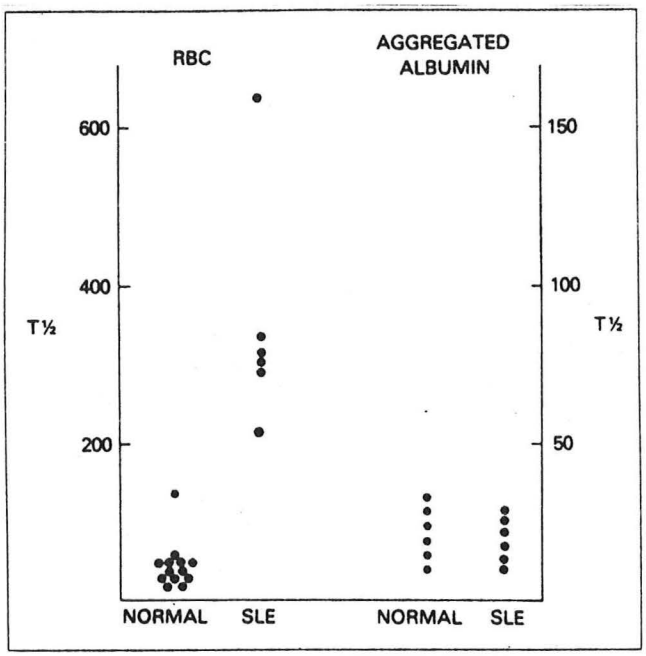


From Haakenstad and Mannik (59)

Figure 14. Relationship between amounts of immune complexes injected and rate of disappearance.

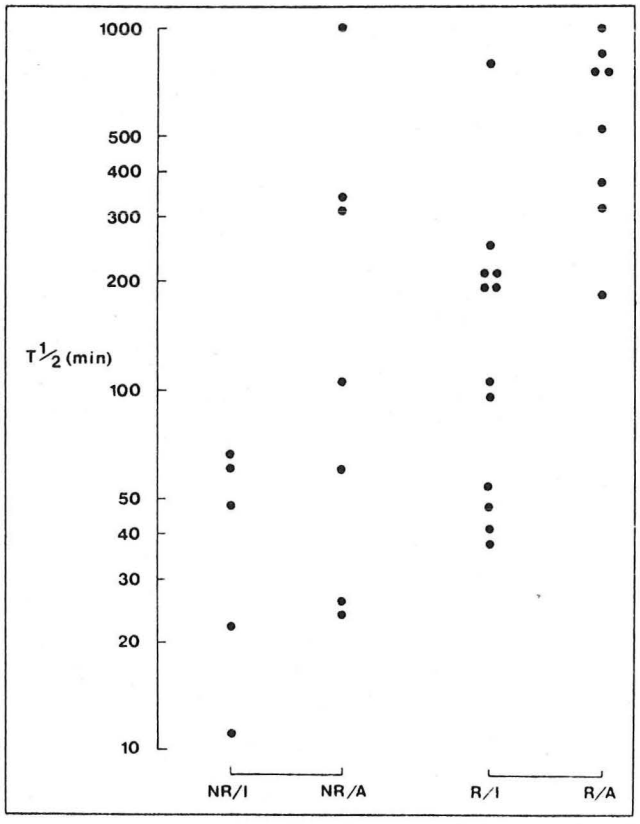
This relationship may have direct therapeutic implications since Barcelli et al (60) have shown that glomerular IC deposition can be decreased by previous activation of the RES. Consideration of this aspect of the natural history of IC is clearly pertinent to the clinical arena since RES dysfunction has recently been demonstrated in several human diseases characterized by the presence of circulating IC (61-65).

In SLE, measurements of immune clearance of antibody-coated erythrocytes have shown a severe dysfunction of the splenic RES system. This abnormality may be present despite apparently normal clearance of non-immune albumin aggregates (Figure 15). The magnitude of this dysfunction is related to general disease activity and varies over time with changes in the degree of activity (66). Moreover, patients with lupus nephritis have more profound defects in clearance than a group of patients with similar disease activity but without renal disease (Figure 16). Thus, it appears that both renal and non-renal disease activity correlate with the magnitude of RES dysfunction.



From Frank et al. (62)

Figure 15. Clearance of immune and non-immune complexes in patients with SLE.



From Parris et al. (66)

Figure 16. Relationship between renal and non-renal disease activity, and clearance of immune complexes in SLE.

Awareness of the importance of RES blockade in the clearance of IC has recently resulted in the development of potentially important therapeutic strategies. The most striking example involves the treatment of idiopathic thrombocytopenic purpura with intravenous gamma globulin (67). This treatment induces a rapid increase in circulating platelet count probably associated with blockade of the Fc receptors of the RES. Conversely, it is likely that the therapeutic effects of plasmapheresis on IC diseases are not merely due to removal of antibodies and complexes from circulation, but also to an improvement in RES-mediated clearance (68).

TABLE 9

Effects of Plasmapheresis in Immune Complex Diseases

1) Removal of Noxious Antibodies
2) Removal of Circulating Immune Complexes
   a) Decrease in tissue deposition
   b) Increase in RES clearance
   c) Modification of the immune response

Methods of Detection of Immune Complexes

The presence of IC has been reported in such disparate conditions as the postprandial state and SLE. Table 10 lists some of the conditions associated with IC. However, this list is far from exhaustive, for new items are being constantly added to the roster. In recent years, the literature has been flooded with large numbers of papers describing new methods for the detection of IC (69,70). From our discussion above, it is clear that no one method may provide adequate information to allow the clinician to correlate the presence, concentration, or characteristics of circulating IC with tissue injury in any given disease. Even in SLE where an IC pathogenesis has been firmly established, different methods of measurement of IC yield different results as to possible correlations with disease activity.

TABLE 10

DISEASES ASSOCIATED WITH IMMUNE COMPLEXES

Autoimmune diseases
Rheumatoid arthritis, Felty's syndrome, systemic lupus erythematosus, Sjögren's syndrome, mixed connective tissue disease, periarteritis nodosa, systemic sclerosis
Glomerulonephritis
Exogenous and endogenous antigens
Neoplastic diseases
Solid and lymphoid tumors
Infectious diseases
Bacterial: Infective endocarditis, meningococcal infections, disseminated gonorrheal infection, recurrent infections in children, infected ventriculoarterial shunt, streptococcal infections, leprosy, syphilis
Viral: Dengue hemorrhagic fever, cytomegalovirus infections, viral hepatitis, infectious mononucleosis, SSPE (subacute sclerosing panencephalitis)
Parasitic: Malaria, trypanosomiasis, schistosomiasis, filariasis, toxoplasmosis
Other conditions
Dermatitis herpetiformis and celiac disease, ulcerative colitis and Crohn's disease, myocardial infarcts, idiopathic interstitial pneumonia, cystic fibrosis, sarcoidosis, multiple sclerosis, amyotrophic lateral sclerosis, myasthenia gravis, uveitis, otitis media, atopic diseases, arthritis associated with intestinal bypass procedure for morbid obesity, sickle-cell anemia, thrombotic thrombocytopenic purpura, primary biliary cirrhosis, kidney and bone marrow transplantation, pregnancy, preeclamptic and eclamptic syndrome, Lyme arthritis, steroid-responsive nephrotic syndrome, xanthomatosis, vasectomy, oral ulceration and Behçet's syndrome, pemphigus and bullous pemphigoid, IgA deficiency, thyroid disorders, ankylosing spondylitis, iatrogenic diseases

From Theofilopoulos and Dixon (69)

Time does not allow for an exhaustive discussion of all the methods currently employed in laboratories around the country for the detection of IC. We will limit this discussion to the description of two of the most useful techniques. Not surprisingly, the assays that seem to yield pertinent information and results relevant to the clinical situation fall under the category of methods that are able to detect complement-fixing IC.

TABLE 11

Methods for Detecting Circulating Immune Complexes

1. Physical Techniques

   Analytical ultracentrifugation
   Sucrose density gradient centrifugation
   Gel filtration
   Ultrafiltration
   Electrophoresis
   Polyethylene glycol (PEG) precipitation
   Cryoprecipitation

2. Methods Based on the Biological Properties of Immune Complexes

   a. Complement techniques

      Microcomplement consumption tests
      Assays based on interaction of IC with purified C1q
      Assays of breakdown products of complement
      Conglutinin radioimmunoassay
      C3 precipitation assay

   b. Antiglobulin Techniques

      Rheumatoid factor tests
      Other antiglobulin tests

   c. Cellular Techniques

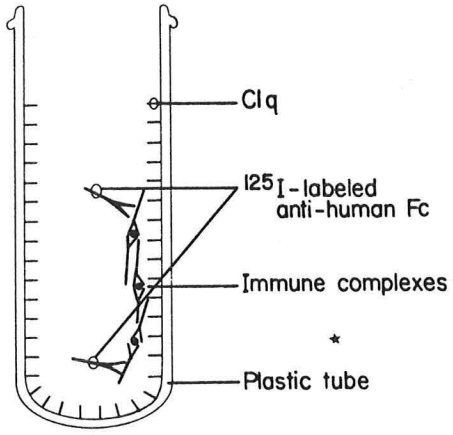
      Platelet aggregation assay
      Inhibition of ADCC
      Intracytoplasmic staining of polymorphonuclear leukocytes
      Enzyme release from eosinophils and mast cells
      Macrophage inhibition assays
      Rosette inhibition assays
      Raji-cell assay
      Human erythrocyte assay

   d. Other Methods

      Binding to Staphylococcal protein A

From Theofilopoulos & Dixon (69)

One of the most popular methods is based on the ability of the first component of complement, Clq, to bind to soluble complexes composed of IgG_1, IgG_2, IgG_3 or IgM. The most reliable method appears to be the solid-phase Clq assay which is described in graphic form in Figure 17 (71).

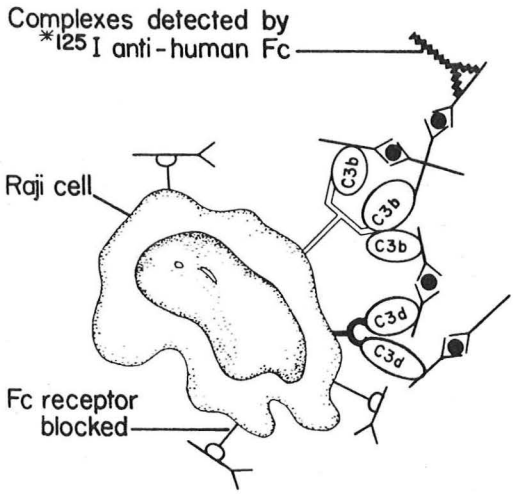


From R.C. Williams, Jr. (70)

Figure 17: Graphic representation of the solid-phase Clq assay.

Purified Clq is bound on the surface of plastic tubes or wells. Test material is then added for a few hours. After washing the loosely bound or free molecules, the IC attached to the Clq are detected by addition of radiolabeled anti-human immunoglobulin antibody. This technique is particularly valuable because it is able to detect small and large molecular weight IC. Its increasing popularity over the classical Clq binding test (72) is due to the smaller proportion of false positive results obtained with the solid-phase Clq test. In the Clq binding test, contaminating DNA or bacterial endotoxin can bind non-specifically to Clq creating artifacts difficult to control.

The second assay which has attained popularity is the Raji-cell test developed by Theofilopoulos et al. (73). The main advantages of this method are its sensitivity and reproducibility. The assay is based on the binding of complement-fixing IC to membrane-bound C3 receptors present on the Raji-cell, a human lymphoblastoid cell line. Since this lymphocyte cell line bears Fc receptors for IgG, these have to be blocked by addition of excess rabbit IgG. The cells are then incubated with the test material so that IC bearing C3 molecules will bind to the C3b cell receptors. The cell-bound IC are then quantitated by incubation with radiolabeled anti-human immunoglobulin antiserum.



From R.C. Williams, Jr. (70)

Figure 18: Principles of the Raji cell test for detection of immune complexes.

The principal drawbacks of this method are 1) the necessity to maintain a cell line in the laboratory, and 2) the potential of obtaining false positive results from the presence in test sera of anti-lymphocyte antibodies. This last point is particularly important because many of the diseases in which IC determinations would be useful are disorders such as SLE, a condition known to be frequently associated with the presence of antibodies to lymphocyte membrane antigens.

CONCLUSION

In this discussion, I have attempted to illustrate with examples drawn from clinical and experimental studies the difficulties encountered in trying to fit the observed facts with the simple paradigm of IC-mediated injury. It is readily apparent that the caveats discussed have led to substantial changes of the simple theory by the addition of a large number of modifiers and additional hypothesis. We may be approaching the time when one such as Frank Dixon or Henry Kunkel, illuminated by a gestalt switch, may propose a new, all-embracing paradigm to keep investigators busy for years to come.

I am grateful to Ms. Debbie McInnis for excellent secretarial help.

REFERENCES

1. Feyerabend P.K. Against Method. Outline of an anarchistic theory of knowledge. Verso. London 1978, p. 55.

2. Williams R.C. Immune Complexes in Clinical and Experimental Medicine. Harvard University Press. 1980.

3. Kuhn T.S. The Structure of Scientific Revolutions. The University of Chicago Press. 1970.

4. Benacerraf B., McCluskey R.T. and Patras D. Localization of colloidal substances in vascular endothelium: A mechanism of tissue damage. I. Factors causing the pathologic deposition of colloidal carbon. Am J Pathol 35:75, 1959.

5. Cochrane C.G. and Koffler D. Immune complex disease in experimental animals and man. Adv Immunol 16:185, 1973.

6. Kniker W.T. and Cochrane C.G. The localization of circulating immune complexes in experimental serum sickness. The role of vasoactive amines and hydrodynamic forces. J Exp Med 127:119, 1968.

7. Cochrane C.G. Studies on the localization of antigen-antibody complexes and other macromolecules in vessels. I. Structural studies. J Exp Med 118:489, 1963.

8. Cochrane C.G. Studies on the localization of antigen-antibody complexes and other macromolecules in vessels. II. Pathogenic and pharmacodynamic studies. J Exp Med 118:503, 1963.

9. Kniker W.T. The role of vasoactive amines in the pathogenesis of complex-induced chronic glomerulonephritis. Fed Proc 27:409, 1968.

10. Kniker W.T. Modulation of the inflammatory response in vivo: prevention or amelioration of immune complex disease. In Inflammation: Mechanisms and Control. I.H. Lepow and P.A. Ward, eds. Academic Press, New York, 1972. p. 335.

11. Chused T.M. and Tarpley T.M. Antagonism of vasoactive amines in NZB/W glomerulonephritis. I. Beneficial effect of methyserzide. Proc Soc Exp Biol Med 144:281, 1973.

12. Kupor L.R., Lowance D.C. and McPhaul J. Single and multiple drug therapy in autologous immune complex nephritis in rats. J Lab Clin Med 87:27, 1976.

13. Kniker W.T., Guerra F.A. and Richards S. Prevention of immune complex disease (serum sickness) by antagonists of vasoactive amines. Ped Res Abstr 5:381, 1971.

14. Fisher E.R. and Bark J. Effect of hypertension on vascular and other lesions of serum sickness. Amer J Pathol 39:665, 1961.

15. Kniker W.T. and Cochrane C.G. Pathogenic factors in vascular lesions of experimental serum sickness. J Exp Med 122:83, 1965.

16. Cochrane C.G. and Janoff A. The Arthus reaction: a model of neutrophil and complement mediated injury. In The Inflammatory Process. B.W. Zweifach, L. Grant and R.T. McCluskey, eds. Academic Press, New York, 1974. V3, p. 86.

17. Atkins R.C., Holdsworth S.R., Glasgow E.F. and Matthews F.E. The macrophage in human rapidly progressive glomerulonephritis. Lancet II:830, 1976.

18. Groggel G.C., Adler S., Rennke H.G., Couser W.G. and Salant D.J. Role of the terminal complement component pathway in experimental membranous nephropathy in the rabbit. J Clin Invest 72:1948, 1983.

19. Mannik M., Arend W.P. and Hall A.P., et al. Studies on antigen-antibody complexes. I. Elimination of soluble complexes from rabbit circulation. J Exp Med 133:713, 1971.

20. Cochrane C.G. and Hawkins D. Studies on circulating immune complexes. III. Factors governing the ability of circulating complexes to localize in blood vessels. J Exp Med 127:137, 1968.

21. Inman R.D. and Day N.K. Immunological and clinical aspects of immune complex disease. Am J Med 70:1097, 1981.

22. Jasin H.E. Scientific overview of systemic lupus erythematosus (SLE). In Therapeutic Apheresis and Plasma Perfusion. R.S.A. Tindall, ed. Alan Liss, Inc., New York, 1982. p. 65.

23. Burnham T.K., Neblett T.R., and Fine G. The application of fluorescent antibody technique to the investigation of lupus erythematosus and various dermatoses. J Invest Dermatol 41:451, 1963.

24. Landry M. and Sams W.M. Systemic lupus erythematosus. Studies of the antibodies bound to the skin. J Clin Invest 52:1871, 1973.

25. Gilliam J.N., Cheatum D.E., Hurd E.R. et al. Immunoglobulin in clinically uninvolved skin in systemic lupus erythematosus. Association with renal disease. J Clin Invest 53:1434, 1974.

26. Koffler D., Agnello V., and Kunkel H.G. Polynucleotide immune complexes in serum and glomeruli of patients with systemic lupus erythematosus. Am J Path 74:109, 1974.

27. Gilliam J.N. The significance of cutaneous immunoglobulin deposits in lupus erythematosus and NZB/NZW F_1 hybrid mice. J Invest Dermatol 65:154, 1975.

28. Feinglass E.J., Arnett F.C. and Dorsch C.A. Neuropsychiatric manifestations of systemic lupus erythematosus: Diagnosis, clinical spectrum and relationship to other features of the disease. Medicine (Baltimore) 55:323, 1976.

29. Atkins C.J., Kondon J.J., Quismorio F.P. and Friou G.J. The choroid plexus in systemic lupus erythematosus. Ann Int Med 76:65, 1972.

30. Bluestein H.G. and Zvaifler N.J. Brain-reactive lymphocytotoxic antibodies in the serum of patients with systemic lupus erythematosus. J Clin Invest 57:509, 1976.

31. Bresnihan B., Oliver M., Grigor R., and Hughes G.R.V. Brain reactivity of lymphocytotoxic antibodies in systemic lupus erythematosus with and without cerebral involvement. Clin Exp Immunol 30:333, 1977.

32. Bluestein H.G., Williams G.W. and Steinberg A.D. Cerebrospinal fluid antibodies to neuronal cells: Association with neuropsychiatric manifestations of systemic lupus erythematosus. Am J Med 70:240, 1981.

33. Hurd E.R., Andreis M. and Ziff M. Phagocytosis of immune complexes by polymorphonuclear leukocytes in patients with Felty's syndrome. Clin Exp Immunol 28:413, 1977.

34. Zivkovic M. and Baum J. Chemotaxis of polymorphonuclear leukocytes from patients with systemic lupus erythematosus and Felty's syndrome. Immunol Commun 1:39, 1972.

35. Gupta R.C., LaForce F.M. and Mills D.M. Polymorphonuclear leukocyte inclusions and impaired bacterial killing in patients with Felty's syndrome. J Lab Clin Med 88:183, 1976.

36. Vincent P.C., Lar J.A. and MacQueen A. The mechanism of neutropenia in Felty's syndrome. Br J Hematol 27:463, 1974.

37. Van Rooijen N. Immune complexes in the spleen. Int Arch Allergy Appl Immunol 49:754, 1975.

38. Hurd E.R., Chubick A., Jasin H.E. and Ziff M. Increased Clq binding immune complexes in Felty's syndrome. Comparison with uncomplicated rheumatoid arthritis. Arthritis Rheum 22:697, 1979.

39. Hecht B., Siegel N., Adler M., Kashgarian M. and Hayslett J.P. Prognostic indices in lupus nephritis. Medicine (Baltimore) 55:163, 1976.

40. Jarrett M.P., Sablay L.B., Walter L., Barland P. and Grayzel A.I. The effect of continuous normalization of serum hemolytic complement on the course of lupus nephritis. A five year prospective study. Am J Med 70:1067, 1981.

41. Lloyd W. and Schur P.H. Immune complexes, complement, and anti-DNA in exacerbations of systemic lupus erythematosus (SLE). Medicine (Baltimore) 60:208, 1981.

42. Appel A.E., Sablay L.B., Golden R.A. et al. The effect of normalization of serum complement and anti-DNA antibody on the course of lupus nephritis. A two year prospective study. Am J Med 64:274, 1978.

43. Rothfield N.F. and Stollar B.D. The relation of immunoglobulin class, pattern of antinuclear antibody, and complement-fixing antibodies to DNA in sera from patients with systemic lupus erythematosus. J Clin Invest 46:1785, 1967.

44. Schur P.H., Monroe M. and Rothfield N.F. The γ G subclass of antinuclear and antinucleic acid antibodies. Arthritis Rheum 15:174, 1972.

45. Sontheimer R.D. and Gilliam J.N. DNA antibody class, sublcass and complement fixation in systemic lupus erythematosus with and without nephritis. Clin Immunol Immunopath 10:459, 1978.

46. Ooi Y.M., Ooi B.S. and Pollak V.I. Relationship of levels of circulating immune complexes to histologic patterns of nephritis: a comparative study of membranous glomerulonephropathy and diffuse proliferative glomerulonephritis. J Lab Clin Med 90:891, 1977.

47. Zager R.A., Couser W.G., Andrews B.S., et al. Membranous nephropathy: a radioimmunologic search for antitubular epithelial antibodies and circulating immune complexes. Nephron 24:10, 1979.

48. Couser W.B. and Salant D.J. In situ immune complex formation and glomerular injury. Kidney Internat 17:1, 1980.

50. Izui S., Lambert P.H. and Miescher P.A. In vitro demonstration of a particular affinity of glomerular basement membrane and collagen for DNA. A possible basis for a local formation of DNA-anti-DNA complexes in systemic lupus erythematosus. J Exp Med 144:428, 1976.

51. Border W.A., Ward H.J., Kamil E.S. and Cohen A.H. Induction of membranous nephropathy in rabbits by administration of an exogenous cationic antigen. Demonstration of a pathogenic role for electrical charge. J Clin Invest 69:451, 1982.

52. Gallo G.R., Caulin-Glaser T. and Lamm M.E. Charge of circulating immune complexes as a factor in glomerular basement membrane localization in mice. J Clin Invest 67:1305, 1981.

53. Jasin H.E. Mechanism of trapping of immune complexes in joint collagenous tissues. Clin Exp Immunol 22:473, 1975.

54. Gelfand M.C., Frank M.N. and Green I. A receptor for the third component of complement in the human renal glomerulus. J Exp Med 142:1029, 1975.

55. Shin M.L., Gelfand M.C., Nagle R.B., et al. Localization of receptors for activated complement on visceral epithelial cells of the human renal glomerulus. J Immunol 118:869, 1977.

56. McPhaul J.J., Jr., and Mullins J.D. Glomerulonephritis mediated by antibody to glomerular basement membrane. Immunological, clinical and histopathological characteristics. J Clin Invest 57:351, 1976.

57. Lerner R.A., Glasscock R.J. and Dixon F.J. The role of antiglomerular basement membrane antibody in the pathogenesis of human glomerulonephritis. J Exp Med 126:982, 1967.

58. Mannik M., Arendt W.P. and Hall A.P. Studies on antigen-antibody complexes. I. Elimination of soluble complexes from rabbit circulation. J Exp Med 133:713, 1971.

59. Haakenstad A.O. and Mannik M. Saturation of the reticuloendothelial system with soluble immune complexes. J Immunol 112:1939, 1974.

60. Barcelli U., Rademacher R., Ooi Y.M. and Ooi B.S. Modification of glomerular immune complex deposition in mice by activation of the reticuloendothelial system. J Clin Invest 67:20, 1981.

61. Kimberly R.P. and Peter R. Endocytosis by the mononuclear phagocyte system and autoimmune disease. Am J Med 74:481, 1983.

62. Frank M.M., Hamburger M.I., Lawley T.J., et al. Defective reticuloendothelial system Fc-receptor function in systemic lupus erythematosus. N Engl J Med 300:518, 1979.

63. Hamburger M.I., Lawley T.J., Kimberly R.P., et al. A serial study of splenic reticuloendothelial Fc receptor functional activity in systemic lupus erythematosus. Arthritis Rheum 25:48, 1982.

64. Hamburger M.I., Moutsopoulus H.M., Lawley T.J., et al. Sjögren's syndrome: a defect in reticuloendothelial system Fc-receptor specific clearance. Ann Int Med 91:534, 1979.

65. Hamburger M.I., Gorevic P.D., Lawley T.J., et al. Mixed cryoglobulinemia: association of glomerulonephritis with defective reticuloendothelial system Fc receptor function. Trans Assoc Am Physicians 92:104, 1979.

66. Parris T.M., Kimberly R.P., Inman R.D., et al. Defective Fc receptor mediated function of the mononuclear phagocyte system in lupus nephritis. Ann Int Med 97:526, 1982.

67. Fehr J., Hofmann V. and Kappeler U. Transient reversal of thrombocytopenia in idiopathic thrombocytopenic purpura by high-dose intravenous gamma globulin. New Engl J Med 306:1254, 1982.

68. Lockwood C.M., Worlledge S., Nicholas A., et al. Reversal of impaired splenic function in patient with nephritis or vasculitis (or both) by plasma exchange. N Engl J Med 300:524, 1979.

69. Theofilopoulos A.N. and Dixon F.J. The biology and detection of immune complexes. Adv Immunol 28:89, 1979.

70. Williams R.C., Jr. Immune complexes in clinical and experimental medicine. Harvard University Press, Cambridge, Mass, 1980. p. 167.

71. Hay F.C., Nineham L.J. and Roitt I.M. Routine assay for the detection of immune complexes of known immunoglobulin class using solid phase Clq. Clin Exp Immunol 24:396, 1976.

72. Nydegger U.E., Lambert P.H., Gerber H., et al. Circulating immune complexes in the serum in systemic lupus erythematosus and in carriers of hepatitis B antigen. Quantitation by binding to radiolabelled Clq. J Clin Invest 54:297, 1974.

73. Theofilopoulos A.N., Wilson C.B., Bokisch V.A., et al. Binding of soluble immune complexes to human lymphoblastoid cells. II. Use of Raji-cells to detect circulating immune complexes in animal and human sera. J Exp Med 140:1230, 1974.