Recent Advances in
RENAL MEDICINE

EDITED BY
NORMAN F. JONES
and
D. K. PETERS

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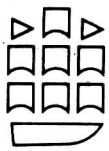
NUMBER TWO



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Preface

This edition differs from its predecessor in a number of respects. First, Professor Keith Peters has joined me as an editor and it is my pleasure to thank him for his stimulating and easy collaboration. Secondly, the list of contributors has been changed almost completely, only the two editors and Professor De Wardener having written in the first edition. The new contributors from Australia, Germany and America are particularly welcome. Finally, there is a shift in emphasis compared with the first edition. While neither edition attempts to be a comprehensive text covering all aspects of Nephrology this second volume tends to consider smaller topics in greater depth than Volume 1.

Those involved in the planning of this book are too numerous to thank by name but this does not diminish my gratitude and indebtedness to them. Mrs Margaret Moore gave unstinting secretarial help and I thank her. It was again a pleasure to work with the representatives of Churchill Livingstone.

London, 1982 N.F.J.

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1. New perspectives in glomerulonephritis

J. S. Cameron D. K. Peters A. J. Rees

INTRODUCTION

In the first edition of this series, published in 1975, the pathogenetic mechanisms in nephritis were reviewed in the light of a substantial body of clinical and experimental data (Peters and Williams, 1975). The important broad distinctions between nephritis mediated by antibodies to renal structures, particularly the basement membranes — examples of type II allergic reactions — and tissue injury brought about by renal deposition of antigen-antibody complexes — type III reactions — had been made and immunofluorescence studies of large series of renal biopsies suggested that immune complex disease accounted for the majority of acute and subacute nephritis, and autoantibodies to glomerular basement membrane for only a tiny minority. Replicating micro-organisms providing a continuing source of antigenic stimulation had been identified in animals and in man, and the idea that certain forms of immunity deficiency by predisposing to chronic infection, or leading to generation of antigen-antibody complexes poorly cleared by the normal mechanisms of the reticulophagocytic system, as a cause of immune complex disease, had been advanced. The clearest illustration of this mechanism was provided by the very rare deficiencies of the complement system which appeared to create a strong predisposition to putative immune complex disease. The principal elements of the immuno-inflammatory processes in the kidney, involving complement, polymorphs and fibrin deposition were also well recognised, and the importance of factors increasing glomerular permeability and thereby favouring glomerular accumulation of antigen-antibody complexes had been stressed by the work of Cochrane and his colleagues (Cochrane and Koffler, 1973).

Various advances have taken place since then. These include: the better recognition of tubular and interstitial disease — i.e. some shift of emphasis from the glomerulus as the target of immunopathological processes (Andres and McCluskey, 1975); evidence which has more directly incriminated type IV — cell mediated allergic reactions in glomerular injury (Schreiner et al, 1980; Bhan et al, 1980); better understanding of the nature of inflammatory cells in glomerulonephritis, especially the role of the macrophage both in crescent formation (Atkins et al, 1978) and mediating capillary injury in acute serum sickness (Holdsworth et al, 1981); a growing appreciation that the immunohistological appearances of granular deposits of Ig and complement may not always result from deposition of soluble circulating antigen-antibody complexes but may reflect reactions between circulating antibody and discrete glomerular antigens (Van Damme et al, 1978). The distinctive alternative pathway activator, the so-called nephritic factor (NF-AP) associated with membrano-proliferative nephritis has been characterised as a remarkable auto-antibody which

acts by stabilising an otherwise labile complement enzyme (Scott et al, 1978). An important consequence of the ideas derived from the experimental work of the 1950s and 1960s has been that emphasis has been placed on the study of circulating immune reactants in patients with nephritis. In the case of autoantibody mediated disease this has been rewarding, but in immune complex disease, in spite of considerable efforts resulting in the availability of various good assays for circulating immune complexes, the position from a nephrologist's point of view is disappointing, for many patients appear not to have circulating immune complexes at the stage they are investigated. Also disappointing is the failure to identify antigens responsible in the vast majority of patients with putative and immune complex disease. Nor has much advance been made in the understanding of the nature and mechanisms underlying the chronic inflammatory processes which are the final common pathway leading to glomerulosclerosis and renal failure in the majority of patients with nephritis.

In this chapter we will not attempt to provide a comprehensive view of the immunopathology of nephritis, but to highlight some important shifts of emphasis. The areas that we will concentrate on are: the immunogenetic basis for certain types of glomerulonephritis in man and in experimental animals; the concept of immune complex disease in the pathogenesis of nephritis in the light of recent clinical and experimental data; and finally, the influence of inherited deficiencies of complement components and of C3 nephritic factors in the development of nephritis.

GENETIC SUSCEPTIBILITY

Little is known of the predisposing factors to nephritis. Interest in immunogenetics has arisen from the recognition of the importance of variation in antibody response in underlying the expression of immune complex disease in experimental systems, the recognition of occasional familial occurrences of glomerulo-nephritis in man, and the current wave of information on HLA associations with other diseases.

HLA SYSTEMS IN GLOMERULONEPHRITIS

Preamble

Some consideration of methods of ascertainment of association and genetic linkage is needed to help to evaluate the array of reports relating disease to HLA.

The demonstration of genetic linkage is readily achieved in family studies, where the relation between the gene product and its associated disease can be tested. This has been the basis of informative studies in for example, diabetes mellitus (Hodge et al, 1981) and auto-immune thyroid disease. The rarity of informative (i.e. diseased) kindreds precludes this approach in glomerulonephritis. The involvement of the MHC must therefore be inferred from association of particular HLA gene products with disease in populations. More rigorous preconditions must be satisfied before such associations can be regarded as being of biological or pathological significance, and a special statistical approach has been developed to deal with these problems (Svejgaard et al, 1974). Generally the biological associations are defined in terms of their strength, that is the proportion of the diseased population to bear the particular HLA gene product, or by the relative risk which is a measure of the increased risk over a control population conferred by the possession of the gene product.

Studies of HLA and disease also require precise definition of disease, otherwise important associations may be lost — the explanation for the failure of early studies of chronic nephritis (Mickey, Kreisler and Terasaki, 1972; Jensen et al, 1975) to identify the immunogenetic linkages that are the subject of this section. Comparison must also be restricted to individuals with same racial backgrounds; and patients should be studied at similar stages in their disease to dissociate genetic influences on susceptibility from genetic influences on progression of disease.

Anti-GBM disease (Table 1.1)

Table 1.1 HLA Antigens in anti-GBM Disease

	Patients	Controls	Relative risk	Pc
DR2	32/36	22/113	33.7	0.22×10^{-8}
B7	21/36	33/153	5.25	0.33×10^{-3}
A3	15/36	43/153	2.4	0.69

A strong association exists between HLA DR2 and anti-GBM disease (Rees et al, 1978). We have found this antigen in 32 of 36 caucasian patients (compared with 22 of 113 controls).

The incidence of HLA B7 is also increased. This was initially interpreted as due to the known linkage (linkage disequilibrium) between DR2 and B7, but more careful analysis showed that patients with HLA DR2 and B7 had a worse prognosis than patients with HLA DR2 not bearing B7 (Rees et al, 1980). This had led to the suggestion that DR2 is a marker for susceptibility to the development of anti-GBM antibodies, but B7 influences the course and severity of disease (see later).

Membranous nephropathy (Table 1.2)

Table 1.2 HLA associations of membranous nephropathy

Author	Country	Number of patients studied	HLA ABC associations Ag	% pt.	% cont.	RR	Pc	HLA D/DR associations Ag	% pt.	% cont.	RR	Pc
Klouda (1979)	U.K	48	B18	21	6			DR3	75	20	12.0	<0.000014
			B8	46	28							
Müller (1981)	GDR	21	B18	0								
			B8	46	20			DR3	76	23	10.7	<0.00001
Histocompatibility Workshop (1980)	Spain	39	B18	46	11		<0.05					
			B8	45	7			DR3	67			<0.001
Histocompatibility Workshop (1980)	USA	33	B18	15	7			DR3	33			NS
			B8	30	15							

Results from the Histocompatibility Workshop are taken from Gavaroy (1980)

Idiopathic membranous nephropathy has been strongly associated with HLA DR3 in the UK and Europe (Klouda et al, 1979; Muller et al, 1981). The results from the USA have been less clear cut (Garovoy, 1980). In the UK, a strong association of

disease with haplotype HLA DR3, B18, BfF1 (the last, a rare complement haplotype encoded in the MHC) has been identified (Dyer et al, 1980) and it appears that the patients bearing this haplotype are less likely to undergo spontaneous remission than patients with membranous nephropathy in which one or more components of this haplotype are lacking (Cairns et al, 1980).

Minimal change nephropathy (Table 1.3)

Table 1.3 HLA associations of minimal change nephrotic syndrome

Author	Country	Number of patients studied	HLA ABC associates					HLA DR associates				
			Ag	% pt.	% cont.	RR	Pc	Ag	% pt.	% cont.	RR	PC
Thomson (1976)	U.K	71	B12	54	15	2.9	<0.02					
			B8	42	28	–	NS					
Trumpeter (1980)	U.K	45										
Alfiler (1980)	Australia	55	B12	33	30	–	NS	DR7	71	30	4.2	<0.005
			B8	36	25	–	NS					
O'Reagan (1980)	Ireland	54	B12	41	37	–	NS					
			B8	65	35	3.5	<0.01					
Mouzon-Campon (1981)	France	54	B12	30	46	–	NS	DR7	67	31	4.4	0.9×10^{-3}
			B8	20	7.7	–	NS					
Noss (1981)	Germany	45	B12	18	22							
			B8	42	21	2.81	<0.027					
			B13	20	4.3	4.65	0.0002					

The occasional occurrence of this disorder in families and in atopic subjects and the various hints for an underlying immunopathogenesis involving products of lymphocytes, led to studies with HLA in this disease. A report of increased incidence of B12 (Thomson et al, 1976) though confirmed in later study in the same unit (Trompeter et al, 1980) has not been substantiated by others (Alfiler et al, 1980; O'Reagan et al, 1980; Mouzon-Campon et al, 1981; Alfiler et al, 1980). However, two recent reports (Mouzon-Campon et al, 1981; Alfiler et al, 1980) indicate an association with HLA DR7 which is known to be in linkage disequilibrium with B12.

Mesangial IgA disease and Henoch Schonlein nephritis (Table 1.4)

Here the picture is confused, for at least 18 studies in 10 countries have given conflicting data (Table 1.4). Early reports of an association of HLA BW35 have not been confirmed, though it is possible that this genetic marker indicates a susceptibility to progressive disease (Kashwabaro et al, 1980). Association with DR4 in France (Fauchet et al, 1980) and Japan (Komori et al, 1979) have recently been reported, but await confirmation.

THE MHC AND EXPERIMENTAL NEPHRITIS

Mercuric chloride induced anti-GBM nephritis in brown Norway rats

Strain susceptibility to develop autoantibodies to GBM following exposure to mercuric chloride has been the subject of considerable work by Druet and his

Table 1.4 HLA associations of mesangial IgA disease

Author	Country	Number of patients studied	HLA ABC association					HLA D/DR association				
			Ag	% pt.	% cont.	RR	Pc	Ag	% pt.	% cont.	RP	Pc
Nyulassy (1977)	Czechoslovakia	29	BW35	28	9	2.8	0.01					
Noel (1978)	France	29	BW35	48	19	4.0	0.005					
Brettle (1978)	UK	17	BW35	18	13	–	NS	DR4	23	33		NS
Bertoux (1978)	France	43	BW35	40	13		NS					
Nagy (1979)	Hungary	24	BW35	4.2	15	–	NS					
Richman (1979)	USA	17	BW35	18	16	–	NS					
			B12	59	20	5.7	<0.05					
Komori (1979)	Japan	40	BW35	10	13.9	–	NS		46	18	3.8	<0.02
Savi (1979)	Italy	23	BW35	9	23	–	NS					
Kashwabara (1980)	Japan	24	BW35	33	12			DR4	67	30	4.79	<0.0016
Bignon (1980)	France	73	BW35	13.7	12.7	–	NS	DR4	14	21	–	NS
Fauchet (1980)	France	45	BW35	27	13	–	NS	DR4	49	20	3.96	<0.0004
Chan (1981)	Hong Kong	34	BW35	3	5	–	NS					
Armaiz-Villena (1981)	Spain	27	BW35	33	22	–	NS					

colleagues (Druet et al, 1977; Druet et al, 1979; Sapin et al, 1981) who have shown the existence of two sets of susceptibility genes. One coded for in the MHC and closely linked with the BN tissue type (RT1-A^n) which interacts with one or two additional genes outside the MHC.

Anti-TBM antibodies in guinea pigs and rats

In guinea pigs the generation of an auto-antibody to tubular basement membrane occurs in strain 13 but not strain two guinea pigs (Hyman et al, 1976) and the only difference between these strains lies in the MHC. By contrast, strain differences in susceptibility to anti-TBM in rats immunised with heterologous basement membranes is not due to failure to generate antibody, but to strain variation in the antigenic structure of rat TBM (Kreiger, Thoenes and Gunther, 1981).

Heymann nephritis

Only certain strains in rats develop nephritis in response to immunisation with autologous renal tubular epithelial cell antigen (RTE). Genetic studies indicate susceptibility is controlled by the MHC (Stenglein, Thoenes and Gunther, 1975; 1978).

Chronic serum sickness and antibody activity

Steward has shown that mice generating high avidity antibody to foreign serum proteins are less likely to develop chronic nephritis than animals generating low avidity antibodies (Devey and Steward, 1980). Genetic studies have shown that the variation in avidity in antibody response is under polygenic control (Steward, Reinhardt and Staines, 1979).

NATURE OF ASSOCIATIONS BETWEEN MHC AND NEPHRITIS

Structure and function of the MHC

In simplest terms, the MHC coded antigens seem to play a critical role in cell to cell interaction that underly the immune response and its regulation. In mice, rats and man, two classes of MHC coded antigen can be distinguished. Class 1 antigens — designated K and D in mice, RT1-A in rats, and HLA AB and C in man — are expressed on all nucleated cells and consist of a polypeptide chain covalently bound to Beta 2 microglobulin. Class I antigens facilitate recognition of infected or otherwise

altered target cells by cytotoxic T lymphocytes. Class II antigens — I antigens in mice, RTI-B antigens in rats, HLA DR and SB antigens in man — have more restricted distribution and are found on a proportion of macrophages and other antigen presenting cells, some activated T lymphocytes and all B lymphocytes. Their function is to facilitate the collaboration between these cells in the generation of an immune response. Also located in the MHC are genes that control synthesis of some but not all complement components; C2, C4 and Bf in man.

MHC antigens exert two different types of genetic control of the immune response — specific and non-specific. Specific immune response genes have been identified within the MHC in many species including mice and man and are usually a function of class II antigens; they determine the ability of an animal to respond to an individual immunogen. Since immunogens generally possess many different antigenic sites (epitopes) the effects of specific immune response genes are only observable under special circumstances, for example when using antigens possessing a single epitope; when the concentration of antigen is so low that only one of its idiotopes (the 'strongest') is able to evoke an immune response; or in the case of autoantigens, when effective tolerance is maintained to all but a few epitopes. Non-specific MHC determined genetic control of immune responses have a general effect on antibody response to many antigens. They have been studied extensively by Biozzi and his colleagues (see Biozzi et al, 1980 for review) who have bred mice to be high and low antibody responders to foreign protein antigens. Of the 10 to 15 genes involved in this variation, in response about 10 per cent of this effect is determined by genes encoded in the MHC (Colambani et al, 1979; Mouton et al, 1979) and a similar proportion is determined by immunoglobulin structural genes (Lieberman et al, 1972).

THE MHC AND ALLERGIC DISEASE

It seems reasonably certain, from studies of experimental (Rose, Kong and Sundick, 1980) and human autoimmune disease (Svejgaard et al, 1980) that disorders in which such autoimmune processes are clearly identified are nearly always strongly associated with particular MHC encoded antigens, i.e. that these antigens create a susceptibility to certain autoimmune disorders. From what has already been mentioned, there is a strong possibility that autoantibodies in MHC associated disease have a highly restricted antigenic specificity; this may ultimately be of therapeutic relevance. The complexicity of the interrelationships between MHC-dependent, other genetically determined characteristics and environmental factors is strikingly illustrated by the development of the lupus-like syndrome following exposure to hydrallazine (Batchelor et al, 1981). The following 'risk' factors for the development of the hydrallazine-lupus syndrome are identified: an environmental factor, i.e. administration of the drug, and its dosage; acetylator status, which is genetically controlled and determines hydrallazine catabolism; sex of the patient; and the possession of DR4. Thus a slow acetylator female taking 100 mg daily of hydrallazine and possessing DR4 had a near 100 per cent chance of developing the lupus-like syndrome.

Precisely how the MHC is related to the development of immunological disease remains unclear. Three principal and not necessarily exclusive hypotheses deserve mention: the first evokes the action of specific immune response genes; the second an MHC determined failure to eliminate microbial infection; and the third, a general

disturbance of immunoregulation as a consequence of certain MHC antigens. The association between HLA DR2 (a class II antigen) and anti-GBM disease might be accounted for by the first hypothesis; the second may have more bearing on the pathogenesis of conditions such as ankylosing spondylitis and reactive arthritis (where the associations appear to be with class I antigens) (Ebringer 1980); and the third might be the explanation for the various associations of DR3, which as well as membranous nephropathy include many other autoimmune diseases but which is also associated in healthy subjects with reduced splenic function and antigen processing (Dausset and Contu, 1980) and abnormalities in T cell subpopulations (Lawley et al, 1981).

The last point worthy of speculation is the basis for the apparently poor prognostic significance of certain haplotypes in patients with anti-GBM disease and idiopathic membranous nephropathy, referred to earlier. The antigens involved have been subject to selective pressures which keep them in 'linkage disequilibrium' in the normal population (Bodmer and Bodmer, 1978) and it seems likely that the selective advantage in usual immunological situations would be the generation of a more effective immune response. Whereas this would normally, for example in response to a microbial invasion, be advantageous, under the exceptional circumstances of an autoimmune disease, the opposite would be the case. The observations that the natural history and response to therapy of certain autoimmune diseases may have an immunogenetic basis has important implications for the planning and analysis of therapeutic trials.

IMMUNE COMPLEXES AND GLOMERULONEPHRITIS: SOLUBLE, INSOLUBLE AND IN SITU

A major attraction of the concept of immune complex disease was its power to account for a large part of the variation in presentation, histopathology and clinical course of nephritis. In the experimental systems a spectrum of histological changes can be induced following exposure to a single purified protein antigen (Germuth and Rodriguez, 1973) and these changes appeared to be dependent on the dose of antigen and the host's immune response to it.

The formation of antigen-antibody complexes is not a simple affair. When an antigen and antibody combine, a variety of immune complexes may form varying in composition from single antigen and antibody molecules, to large lattice complexes consisting of many antigen-antibody molecules (Mannik, 1980). With increasing lattice formation complexes become less soluble and eventually precipitate because of their mass, and through the masking of polar groups. All classes of antibody are capable of insoluble immune complex formation since they possess two or more antigen-binding sites; however, only multivalent antigens can unite with antibody to form immune complexes with the high degree of lattice necessary for immune precipitation.

The rate of immune precipitation or insoluble complex formation depends on the relative proportion and properties of the reactants, the temperature, salt concentration and pH of the reaction medium. As indicated, the molar ratio of multivalent antigen antibody has a direct effect on the degree of lattice formation. In antibody excess, insoluble complex formation occurs and increases with increasing concentration

of antigen, achieving a maximum at optimal proportions of antigen to antibody sites. This is called a point or zone of equivalence. Addition of excess antigen beyond this point of equivalence leads to the formation of *soluble immune complexes* and as the concentration of excess antigen increases, insoluble complex formation decreases until only soluble complexes are formed. To what extent these considerations, worked out in vitro, may operate in vivo is not known, especially when immune complexes are constantly being added to and removed from the system so that equilibrium is never established. Certainly, there must be a zone between dissolved and precipitated complexes in which colloidal complexes are found (Seiler and Gronski, 1979).

Other calculable properties which effect the rate of insoluble immune complex formation are the affinity of the antibody and the avidity of the antiserum toward a multivalent antigen. Affinity is the intrinsic association constant of an antibody toward an antigen measured at equilibrium, and it measures the binding strength and stability between antigen and antibody. In the majority of clinical and animal experimental studies, avidity rather than affinity has been reported. The avidity measures the binding strength of an antiserum toward a multivalent antigen, and depends on the affinity of various antibodies present in the antisera. on the pattern of antigenic determinants and on the physical chemical properties of the medium. The higher the affinity or antiserum avidity, the greater the likelihood of insoluble immune complex formation, whilst low avidity antibody gives equivalence at much higher range of antigen concentrations.

Recent work, (Nussenzweig et al, 1975; Schifferli et al, 1981), has also shown that the complement system has an important effect on the antigen-antibody lattice. The classical complement pathway components, by binding to the immune complex can inhibit its precipitations, i.e. inhibit lattice formation; whilst alternative pathway activation results in deposition of C3 on to an immune precipitate, with interposition of C3 molecules into the antigen-antibody lattice, leading to its disruption and eventually dissolution. The stoichochemical and biological properties of antigen antibody complexes are thus subject to substantial modification.

The evidence supporting the idea that soluble complexes are the pathogenetic agent in most forms of glomerulonephritis has been reviewed by a number of authors (Cochrane and Koffler, 1973; Wilson and Dixon, 1981) who have contributed much of the data upon which this hypothesis is based. Like all good hypotheses, descriptions have now been challenged. Couser and Salant (1980) remind us that until 1950 the immune complex hypothesis was not popular, although articulated in a primitive form as early as 1908 by Von Pirquet. Until 1950, most immunologists supposed that tissue injury could only occur at the site of active combination between antigen and antibody (Hawn and Janeway, 1947) by analogy with the Arthus reaction (Opie, 1924).

During the next 15 years, however, a number of workers, in particular Germuth and colleagues (Germuth 1953; Germuth and Rodriguez, 1973) and Dixon and his co-workers (Dixon et al, 1958; Dixon, Feldman and Vasquez, 1961; Dixon 1968), provided compelling evidence that glomerular injury in acute serum sickness nephritis in rabbits resulted from the formation, circulation and tissue deposition of immune complexes formed from the foreign protein (bovine serum albumin in almost all studies) and specific host antibody directed against it. Moreover, they showed that the antibody-antigen system remained in antigen excess throughout the induction and

appearance of the disease, free antibody only being observed after the elimination of immune-complexed antigen from the circulation and the appearance of tissue injury. The analogies, both clinical, histological and immunohistological between the 'one shot' single large injection of foreign protein and acute post-infectious (particularly post-streptococcal) glomerulonephritis in humans were many (Michael et al, 1966) and it is usually postulated that some similar mechanism involving streptococcal or other microbial antigens is operating in the human disease. The subepithelial site of immune deposits ('humps') in both the human disease and the animal model was supposed to arise from penetration of the preformed complexes through the basement membrane.

The correspondence of the chronic models of serum sickness in rabbits and human disease are less immediate, but still strong. Depending upon the response of the animals and the immunisation schedule, various histological and immunopathological appearances were seen, which mimicked most of the forms of glomerulonephritis observed in human disease. Further, Germuth and his colleagues (1967, 1972) were able to demonstrate in rabbits given constant daily doses of a foreign protein (bovine serum albumin) that the animals responded within a week or two in four main ways: first, little or no antibody was produced and no renal disease was observed; second, a high antibody titre was observed and again no renal disease developed; third, a poor antibody titre was seen and mesangial glomerulonephritis was found; and lastly, those animals who from the start had, or later developed a poor immune response in moderate antibody excess, developed severe nephritis. Later Germuth et al (1972) showed that the third group had relatively large soluble immune complexes in the circulation (molecular weight $0.5–1.0 \times 10^{-6}$ daltons) whilst the fourth group had smaller immune complexes (0.30×10^{-6} daltons). Germuth et al reasoned that these immune complexes in the circulation were the pathogenetic agent, but no proof of this was provided, then or since. In Dixon's experiments (1961) ascending doses of BSA were given daily to rabbits in an attempt to match their antibody response assessed weekly. These workers interpreted the glomerulonephritis induced as being the result of soluble immune complexes formed in antigen excess, and these interpretations have been accepted by most observers.

Difficulties with the current hypothesis

Several sets of observations, however, conflict with the idea of already-assembled soluble circulating complexes formed in antigen excess as the pathogenetic agent in all forms of acute and chronic glomerulonephritis.

Injection of preformed immune complexes

The first anomaly was the results obtained when complexes of various types are assembled in vitro and injected into experimental animals. One might expect to reproduce glomerulonephritis with such experiments. These experiments were performed in rabbits by Germuth in 1953, and later by McCluskey, Benacerraf and colleagues (McCluskey et al, 1959, 1960); these and subsequent experiments are detailed in Couser and Salant (1980). Several anomalous results appeared.

First, in rabbits there was no (or almost no) localisation into the glomeruli at all, whatever the size or type of complex used. Subsequent work in rabbits established that a coincident increase in capillary permeability is necessary to induce localisation