

THE SEVENTH K PRATHAP MEMORIAL LECTURE
The pathogenesis of autoimmune immune complex disease

Peter J LACHMANN, FRCPath, FRS

The Seventh K Prathap Memorial Lecture was delivered by Professor Lachmann during his visit to the Malaysian Society of Pathologists in November 1991 as President of the Royal College of Pathologists, United Kingdom

INTRODUCTION

Although the first autoimmune disease - the haemolytic anaemia associated with the Donath-Landsteiner autoantibody, has been known since 1902,¹ the recognition that autoimmunity is a common cause of a wide variety of diseases really stems from recognition of the autoimmune nature of systemic lupus erythematosus (SLE) between 1948 and 1954 and the description of autoimmune thyroiditis at approximately the same time.^{2,3} In the intervening forty years an extensive number

of autoimmune diseases have been described and a great deal has been published on the origins of autoimmunity and on the aetiology and pathogenesis of the diseases concerned. However, it must be admitted that in very few cases is a complete explanation of autoimmune disease available. It has, however, become abundantly clear that autoimmunity is not a single phenomenon but that there are a variety of quite separate mechanisms that can lead to autoimmune reactions and autoimmune disease (Table 1).

TABLE 1: Autoimmune disease

Type	Examples	Proposed aetiological mechanism	Genetic association	
Anti - receptor antibody mediated	Myasthenia gravis	"Internal image" Idiotypic network derangement	(female only) A1 B8 DR3	
	Grave's disease			
"Sequestered antigen"	goodpasture's disease	Abnormal release of antigen	DR2	
	Phaco-anaphylaxis			
Autoimmune disease from mitogenic	Rheumatic fever Reactive Arthritides	Antigenic mimicry between self antigen and infecting pathogen	B27	
				solvent exposure (or infection) surgery
				Strep pyogenes & heart muscle
				Enteric pathogens (Shigella Chlamydia Klebsiella & B27)
Autoimmune endocrino-pathies	IDDM	Aberrant Class II MHC expression on hormone producing cells	A1 B8 DR4 & DR4	
	Hashimoto's disease			
"T-cell" mediated disease	Multiple sclerosis/EAE	Unknown	DR2	
	Rheumatoid arthritis	?Response to unknown infectious agent	DR4	
Autoimmune immune complex disease	Systemic L E Glomerulonephritis	Defect in immune complex handling	Complement deficiency	

Address for correspondence and reprint requests: Professor P. J. Lachmann, Molecular Immunopathology Unit, Medical Research Council Centre, Hills Road, Cambridge CB2 2QH, United Kingdom

It is the last category on Table 1, that of autoimmune immune complex disease, which is to be the subject of this lecture. The prototype disease in this group is SLE, where autoantibodies are formed to a substantial number of non-organ specific intracellular cell components, noticeably the components of the DNA nucleoprotein particle and where immune complexes involving these antibodies and their respective antigens are largely responsible for the pathogenesis. The genetic predisposition to this group of diseases can be found in deficiencies of the early components of the classical pathway of the complement system (Table 2).⁴

Subjects who have homozygous deficiencies of C1 or C4 have an incidence of autoimmune immune complex disease close to 100%. Those deficient in C2 appear to have an incidence of more than 50%, although this may be high since C2 deficiency is relatively common and clinically normal subjects with C2 deficiency are probably under-reported in the literature. Presumably, patients with C2 deficiency are less affected than those with C1 and C4 deficiency because they can fix C4 on their immune complexes and bound C4 shares many of the activities of bound C3. It is interesting that C3 deficient subjects tend to suffer from infections rather than immune complex disease, although a vasculitic illness is not uncommon and it may be that failure to fix C3 by either the classical or the alternative pathway interferes with the mediating mechanisms of the disease as well as those that normally prevent it.

Although the incidence of SLE among patients with homozygous deficiencies of the classical pathway complement components is so high, such patients make up a trivial percentage of patients with SLE. However, when it became apparent that heterozygous deficiencies of C4, or at least of one of its isotopes was relatively common, surveys were done to investigate the frequency of partial C4 deficiency among patients with the disease. It is now clear that not only

in Caucasians but also in Orientals and in American blacks, there is a significant increase in the incidence of C4A null alleles among patients with SLE; indeed more than half such patients carry at least one C4A null allele compared to between 20 and 30% of the various normal populations.^{5,6}

It has long been known that complement plays a role in the mediation of immune complex inflammation and the association of complement deficiency with its occurrence was therefore surprising. The association is physiological and not due to genetic linkage since the genes for C1 and C4 are not on the same chromosome and even the secondary deficiencies of C2 and C4 found in hereditary angioedema carry an increased incidence of SLE.

THE CONSEQUENCES OF COMPLEMENT DEFICIENCY THAT MAY PREDISPOSE TO IMMUNE COMPLEX DISEASE

Effect on antibody formation

There is extensive work on animals demonstrating that deficiencies of the early classical complement components and of C3 impair antibody responses, especially to low doses of antigen given without adjuvants.⁷⁻¹⁰ It has recently been demonstrated that in C4 deficient guinea pig such responses can be reconstituted by the passive administration of human C4A but not human C4B.¹¹ The two human isotopes differ in that C4A preferentially makes amide bonds and therefore binds particularly to immune complexes whereas C4B preferentially forms ester bonds and binds particularly to carbohydrates and cell surfaces. This suggests that not only predisposition to SLE but also the effect on antibody formation is mediated by that isotope of C4 that binds to immune complexes. It was long ago demonstrated by Klaus and Humphrey (1977)¹² that an important mechanism for the role of complement in the immune response is its requirement on immune complexes for the binding

TABLE 2: Complement deficiencies of the early classical pathway components (from Lachmann⁴)

Component	Chromosomal locus	Number	Healthy	I/C disease	(SLE)	Infections (Neisseria)	Other
C1q	1p	17	2	15	(7)	5	(2) Skin lesions
C1r/C1s	12	9	2	7	(6)	-	-
C4	6p	16	2	14	(12)	6	-
C2	6p	77	15	43	(23)	30	(3) -
C1 - Ina	11	>500	-	2 - 5%		? (not reported)	hereditary cedema (all)

of antigen and this takes place in the germinal centres of lymph nodes, the site at which B cell memory is generated.

In humans, there is no overall antibody defect in patients with these early complement component deficiencies but it has been demonstrated that they do have lower IgG4 levels than normal.¹³ C3 deficient patients also have some defect in IgG2 formation. IgG2 is the penultimate, and IgG4 the ultimate IgG subclass formed in the immune response and these deficiencies presumably reflect a difficulty in generating B cell memory. It is therefore possible that the failure to generate these final isotypes may play some role in the pathogenesis of immune complex disease. However, IgG4 does not itself fix the classical pathway of complement or indeed the alternative pathway and it is not immediately apparent how this defect would lead to immune complex disease.

The more likely mechanism concerns the "handling" of the immune complexes.

It was known to Heidelberger some fifty years ago that in the presence of complement, immune precipitation was delayed and the precipitates formed were finer and non-flocculent.¹⁴ This phenomenon was rediscovered in 1975 by Miller and Nussenzweig¹⁵ who showed that preformed immune complexes could be resolubilised in the presence of serum providing the alternative complement pathway was intact. If, however, this experiment is done in a more physiological way by forming the immune complexes in the presence of complement, it is apparent that it is the classical pathway that is required to keep the complex soluble."

The explanation for this effect of complement on immune precipitation is not intuitively obvious and has been explained in terms of an inhibitory effect by fixed complement on Fc-Fc interactions. It seems more likely, however, that the effect can be explained in terms of the Goldberg hypothesis of immune precipitation.^{17,18} This states that the composition of immune complexes can be predicted solely from knowledge of the concentrations of antigen and antibody and the valency of each. The complex most likely to form is that which can be formed in the maximum number of different ways. The mathematics involved are extremely complex, but they predict that the likelihood of precipitation falls sharply as the valency of the antigen is reduced and as the valency of antibody is reduced from two towards one. It seems highly probable that the binding of C4 and C3 onto the antigen antibody lattice (and it is known that C3 binds preferentially to the Fd portion of antibody molecules) will reduce the

valency of antibody molecules that are already bound by one valency and that this will lead to an effective valency drop from two downwards. This change will tend to favour the formation of a large number of small complexes at the expense of a small number of large complexes and account for the failure of precipitation seen. This effect may be important in the pathogenesis of immune complex disease, since very small complexes are not apparently capable of inducing inflammatory disease. Large insoluble immune precipitates do not enter the circulation and can therefore cause inflammation only locally and it is only the complexes of intermediate size that give rise to immune complex disease. What this size is, obviously, depends on the nature of the antigen, but in the experiments of Cochrane and Hawkins¹⁹ it was around 19s. In the presence of an intact complement system this critically sized fraction of immune complexes may be reduced in amount.

A second phenomenon of perhaps even greater importance is whether the immune complexes that are potentially pathogenic are carried in the circulation free in the plasma or whether they are carried bound to red cells through the complement receptor CR1. Immune complexes free in the circulation may come into contact with endothelial surfaces across which they are transported and where they can then give rise to inflammation. This may occur in the kidney, the lungs, skin, or joints. Immune complexes bound to erythrocyte CR1 will, providing there is streamline flow in the vessels, not come into contact with the endothelia and will be removed in the sinusoids of the reticulo-endothelial system where the red cells come into contact with the macrophages lining the sinusoids. Here, the immune complexes will be transferred to receptors on the macrophages (CR1, CR3 and Fc receptors) and here it is believed that CR1, which is highly sensitive to proteolysis, will be proteolysed by macrophage enzymes.

Removal of antigens in the reticulo-endothelial system, perhaps particularly in the liver, allows them to be removed without feedback antibody formation. Where immune complexes are removed in the periphery, an inflammatory reaction ensues with liberation of the autoantigens and the opportunity to make further autoantibody, thus establishing a vicious circle which leads to the formation of immune complex disease. This pathogenesis is shown in Fig 1.

Some of the earlier evidence for such a scheme was the observation that CR1 levels on erythrocytes of patients with SLE were reduced in number proportional to disease activity. The observa-

tion originally gave rise to controversy in that there is some genetic association of CR1 numbers and it was originally believed that low CR1 numbers may predispose to the disease.^{20,21} However, this does not appear to be the case, since the genetic level of CR1 numbers on erythrocytes can be predicted from an RFLP polymorphism in the CR1 gene²² and low levels are found for each phenotype. Furthermore, the average risk for SLE associated with the low allele for CR1 is below 1, so that there appears to be no increased risk of developing disease on the basis of genetic CR1 numbers.²³ Recently, we have been able to raise antibodies that react specifically with a neoantigen exposed in CR1 after proteolysis²⁴ and it can be shown that there is some elevation of levels of proteolysed CR1 on the erythrocytes in a group of patients with SLE.

Furthermore, studies of a C2 deficient patient with SLE who was substantially improved by infusion of fresh plasma²⁵ have shown that this improvement is associated with changes in immune complex clearance, notably increased uptake of immune complexes in the spleen and a more prolonged retention of immune complexes in the liver. Experiments by the same group on the handling of artificial immune complexes made with hepatitis B virus antigens and immune serum have shown that patients with SLE, whether or not they are genetically complement deficient, have shown reduced splenic uptake and reduced liver retention of immune complexes compared with normal subjects (data from K Davis and M J Walport, personal communication). The *in vivo*

data is therefore accumulating about the abnormalities of immune complex clearance.

The antibody response in SLE is antigen driven

If the pathogenic scheme outlined in Fig 1 is correct, it must follow that the autoantibody response in SLE is driven by antigen rather than by the idiotype network or by polyclonal activation. I think that this view is no longer disputed, partly because the spectrum of specificity of antibodies found is clearly related to antigenic particles, notably the DNA-nucleo protein particle, to all components of which (DNA, histone, the native DNA histone complex and non-histone proteins) antibodies are found. Further evidence for this point of view is found in that antigen presentation appears to be required for the formation of such autoantibodies since interference with antigen presentation in mice with SLE by infecting them with LDH virus (which apparently uses Class 2 MHC as a receptor and kills Class 2 positive macrophages) leads to arrest of formation of nuclear antibodies.²⁶

A further requirement from the pathogenic scheme outlined is that the formation of at least small amounts of such autoantibodies should not be regarded as essentially abnormal, for it is the failure to eliminate the complexes and prevent the establishment of feedback cycles which seems to be important. In this connection it is interesting that there are studies as long ago as 1967²⁷ which show that the formation of low levels of IgM antibodies to nuclear antigen are common follow-

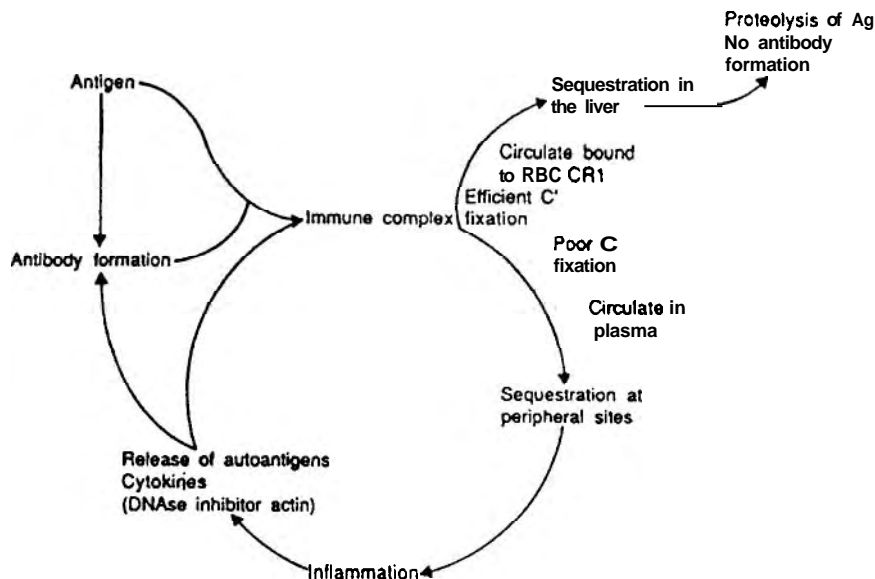


FIG. 1: Pathogenesis of immune complex disease (from Lachmann⁴)

ing pulmonary infarction. In all such patients the antibodies had totally disappeared again four weeks later.

Summary

It has been proposed that autoimmune immune complex disease, of which SLE is the type example, is caused essentially by a failure to properly metabolise immune complexes and that this allows the establishment of feedback cycles which cause more immune complexes to be formed. The essential genetic predisposition to this disease is complement deficiency of the components of the early classical pathway and some degree of genetic complement deficiency, particularly of C4a, is found in more than half the patients. It seems likely that acquired complement deficiencies, possibly present at the time of initiation of the disease, may be important in many of the other cases.

REFERENCES

1. Donath J, Landsteiner K. Wien k Rundschau 1902; 40.
2. Roitt IM, Doniach D, Campbell PN, Vaughan Hudson R. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). Lancet 1956; ii: 820-1.
3. Witebsky E, Rose NR, Terplan K, Paine JR, Egan RW. Chronic thyroiditis and autoimmunization. JAMA 1957; 164: 1439-47.
4. Lachmann PJ. Complement deficiency and the pathogenesis of autoimmune immune complex disease. Chemical Immunology 1990; 49: 245-63.
5. Fielder AHL, Walport MJ, Batchelor JR, Rynes RI, Black CM, Dodi IA, Hughes GRV. Family study of the major histocompatibility complex in patients with systemic lupus erythematosus: importance of null alleles of C4A and C4B in determining disease susceptibility. Br Med J 1983; 286: 425-8.
6. Dunckley H, Gatenby PA, Hawkins B, Naito S, Serjeantson SW. Deficiency of C4A is a genetic determinant of systemic lupus erythematosus in three ethnic groups. J Immunogenet 1987; 14: 209-18.
7. Ellman L, Green I, Judge F, Frank MM. In vivo studies in C4-deficient guinea pigs. J Exp Med 1971; 134: 162-76.
8. Frank MM, May J, Gaither T, Ellman L. In vitro studies of complement function in sera of C4-deficient guinea pigs. J Exp Med 1971; 134: 176-87.
9. Pepys MB. Role of complement in the induction of the allergic response. Nature New Biol 1972; 237: 157-9.
10. Burger R, Gordon J, Stevenson G, Ramadori G, Zanker B, Hadding U, Bitter-Suermann D. An inherited deficiency of the third component of complement, C3, in guinea pigs. Eur J Immunol 1986; 16: 7-11.
11. Finco O, Li S, Cuccia M, Rosen FS, Carroll MC. Structural differences between the two human complement C4 isotypes affect the humoral immune response. J Exp Med 1992; 175: 537-43.
12. Klaus GGB, Humphrey JH. The generation of memory cells. I. The role of C3 in the generation of B memory cells. Immunology 1977; 33: 31-40.
13. Bird P, Lachmann PJ. The regulation of IgG subclass production in man: low serum IgG4 in inherited deficiencies of the classical pathway of C3 activation. Eur J Immunol 1988; 18: 1217-22.
14. Heidelberger M. Quantitative chemical studies on complement or alexin. J Exp Med 1941; 73: 681-94.
15. Miller GW, Nussenzweig V. A new complement function: solubilization of antigen-antibody aggregates. Proc Natn Acad Sci USA 1975; 72: 418-22.
16. Schifferli JA, Peters DK. Complement, the immune-complex lattice, and the pathophysiology of complement-deficiency syndromes. Lancet 1983; i: 957.
17. Goldberg RJ. A theory of antibody-antigen reactions: I. Theory for reactions of multivalent antigen with bivalent and univalent antibody. J Am Chem Soc 1952; 74: 5715-25.
18. Goldberg RJ. A theory of antibody-antigen reactions: II. Theory for reactions of multivalent antigen with multivalent antibody. J Am Chem Soc 1953; 75: 3127-31.
19. Cochrane CG, Hawkins D. Studies on circulating immune complexes. III. Factors governing the ability of circulating complexes to localise in blood vessels. J Exp Med 1968; 127: 137-54.
20. Miyakawa Y, Yamada A, Kosaka K, Tsuda F, Kosugi E, Mayumi M. Defective immune adherence receptor (C3b) on erythrocytes from patients with systemic lupus erythematosus. Lancet 1981; ii: 493-7.
21. Wilson JG, Wong WW, Schur PM, Fearon DT. Mode of inheritance of decreased C3b receptors on erythrocytes of patients with systemic lupus erythematosus. N Engl J Med 1982; 307: 981-6.
22. Wilson JG, Murphy EE, Wong WW, Klickstein LB, Weis JH, Fearon DT. Identification of a restriction fragment length polymorphism by a CR1 cDNA that correlates with the number of CR1 on erythrocytes. J Exp Med 1986; 164: 50-9.
23. Moldenhauer F, David J, Fielder AHL, Lachmann PJ, Walport MJ. Inherited deficiency of erythrocyte complement receptor type 1 (CR1) does not cause disease susceptibility to systemic lupus erythematosus. Arth Rheum 1987; 30: 961-6.
24. Barbosa JE, Harrison RA, Barker PJ, Lachmann PJ. An anti-peptide antibody that recognizes a neo-antigen in the CR1 stump remaining on erythrocytes after proteolysis. Clin Exp Immunol 1992; 87: 144-9.
25. Steinsson K, Erlendsson K, Valdimarsson H. Successful plasma infusion treatment of a patient with C2 deficiency and systemic lupus erythematosus: clinical experience over forty-five months. Arth Rheum 1989; 32: 906-13.
26. Oldstone MBA, Dixon FJ. Inhibition of antibodies to nuclear antigens and to DNA in New Zealand mice infected with lactate dehydrogenase virus. Science 1972; 175: 784-6.
27. Biro CE. Algunos conceptos inmunologicos en relacion con la patogenia del lupus eritematoso diseminado. Arch Inst Cardiol Mex 1967; 37: 669-74.