DEOXYRIBONUCLEIC ACID AND ITS ANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

Chee Yam Cheng

SYNOPSIS

Deoxyribonucleic acid (DNA) and the antibodies directed against it are closely associated with systemic lupus erythematosus (SLE) and there is strong evidence that they are pathogenetic. Titres of these antibodies not always correlate with disease severity or activity. Antibodies to double-stranded DNA (dsDNA) are specific for SLE unlike those to single-stranded DNA. Present-day tests for dsDNA antibody estimation must ensure that the exogenous source of DNA used as antigen for detection of its antibody is free of single-stranded contamination. Three newer tests - the Crithidiae luciliae kinetoplast, synthetic poly dAT and the bacteriophage PM2DNA - are mentioned.

Qualitative variables of DNA antibodies, such as their avidity, complement-fixing ability, immunoglobulin class and subclass, their interaction in immune complex formation, and the role of the reticuloendothelial system are examined to try and explain the differences in clinical manifestations between SLE patients (lupus subsets) and also within the same patient but at differing stages of the disease. The modifying influence of the extractable nuclear antigen (ENA) and their antibodies (to ribonucleoprotein (RNP) and Sm antigen) is highlighted. Finally there is discussion on the immuno-regulatory dysfunction involving the IgG and IgM classes of anti-DNA antibody. The cellular aspects of immuno-regulation have been omitted.

INTRODUCTION

Deoxyribonucleic acid (DNA) is basically a double-stranded molecule and antibodies directed against this "native" double-stranded DNA (dsDNA) were discovered in sera of patients with systemic lupus erythematosus (SLE) more than 20 years ago (1, 2). These anti-dsDNA antibodies are important in the pathogenesis of SLE and their levels correlate with disease activity and active nephritis (3-5). Detecting these dsDNA antibodies in patients'

Department of Medicine III
Tan Tock Seng Hospital
Singapore

Chee Yam Cheng, MBBS, MRCP (UK), M Med (S'pore)
S. Registrar

sera is now routine in most clinical laboratories. In the plethora of techniques that have been employed, e.g. gel diffusion, complement fixation, agglutination, radioimmunoelectrophoresis, counter-immunoelectrophoresis and ammonium sulphate precipitation (Farr assay), an exogenous source of DNA, whose purity for double-strandedness is not always assured, is used. Metabolic processes such as replication, transcription and repair temporarily disrupt the double-stranded nature of DNA. Handling of the DNA prior to its use in the actual estimation of anti-dsDNA antibody level can and does result in shearing the DNA molecule exposing small single-stranded (ss) regions on the ends or sides of the large ds molecule. Replication and recombination cause the formation of internal singlestranded regions during intermediate stages. For these reasons, the most carefully prepared DNA from bacterial and eukaryotic DNA usually shows 10-20% of the total supposedly dsDNA as having ss regions (6). Work by Arana and Seligman (7) suggests that many SLE sera contain at least three types of antiDNA antibodies.

(1) antidsDNA antibodies that react only with "native" dsDNA
(2) antidsDNA antibodies that react with "native" dsDNA but cross-react with denatured or incompletely dsDNA.
(3) antissDNA antibodies.

Some antibodies in SLE sera are directed against purine or pyrimidine bases while others appear to be directed against the sugar-phosphate "backbone". Workers have showed that antibodies to dsDNA may recognise the antigenic determinants involving the external deoxyribose phosphate "backbone" in contrast to the internal nucleoside-specific determinants said to be sterically protected in dsDNA but exposed and detectable in ssDNA (7, 8).

DNA antibodies in sera and tissues

DNA is not the only antigen to which antibodies are found against in SLE. Brentjens et al (9) showed immune complex deposits in vasculitic lesions in SLE in organs like the kidneys, lungs, spleen, liver, intestines, peritoneum and the choroid plexus. The composition of these immune complexes especially in lupus nephritis has been identified with both DNA and its antibody being present in eluates from lupus nephritic renal tissue (10). Primary involvement of DNA/antiDNA complexes in the development of renal injury in SLE has been further established (4, 11-13) and increased serum levels of antidsDNA and to a lesser extent antissDNA correlate closely with clinical activity and hypocomplementaemia (4, 11, 12). Both dsDNA (10) and ssDNA (14) have the same distribution along the glomerular basement membrane as gamma globulin and complement components. Accummulating evidence favours dsDNA antibodies as being specific for SLE and do not just reflect antibodies to denatured and single stranded regions in the test material. Locker et al (6) concluded that non-SLE sera do not contain antibodies specific for dsDNA at levels comparable to those found in SLE sera, but rather contain high levels of antibodies reacting with ss regions or mixed DNA.

Tests for DNA antibody

SLE patients' sera react with DNA irrespective of whether the DNA is of plant, viral, bacterial or mammalian source. However, sera from one patient when tested against DNA from these various sources gave a % binding ranging from 5 to 95% in the Farr assay (15). This was ascribed to variations in the molecular weight of the DNA; the higher the molecular weight of DNA, the greater the binding.

Thus the heterogeneity of molecular size of the DNA and its purity for ds are very important considerations in any test used to detect dsDNA antibodies. False positive results can easily result from ssDNA contamination.

To circumvent these problems of the DNA antigen, the dsDNA of the haemo-flagellate, Crithidiae luciliae kinetoplast (16), synthetic dAT (17) and circular viral DNA (18) have been used. With the same sera run on these supposedly pure dsDNA antigens, the results would hopefully be more specific for SLE. However, some problems have arisen.

With the Crithidiae assay, which is now available in a kit form commercially, indirect immunofluorescence does also detect serum antibodies that react with a non-kinetoplast non-nuclear antigen present in the regions of the basal feet associated with the basal body of the organism's flagellum (19). Unless one is aware of this possibility, this immunofluorescence could be misinterpreted as positive for dsDNA antibody. The significance of this antibasal feet antibody is unknown. It was found in 16 sera of 31 patients with SLE (19).

Synthetic dAT is alternating copolymers of deoxyadenate and deoxythimidylate. Steinman et al (20) found that high concentrations of antibodies to this dAT, using the % binding assay, correlated with high incidence of active renal lupus in patients. Proceeding further, Heinzerling and co-workers (21) found that only sera containing a high concentration of IgG antibody to DNA would bind to dAT. Thus it appeared that the selectivity of IgG antibody for dAT correlated with IgG being the main antibody type involved in lupus nephritis.

Supercoiled, circular viral DNA is isolated from the pseudomonas bacteriophage PM2. Though not very stable, it does have extraordinarily high specificity for the dsDNA antibody in active SLE. Another advantage is that variations in molecular weight of the DNA are excluded. Such variations give rise to enormous fluctuations of dsDNA binding values (22, 23).

Therefore although these three DNA sources may offer advantages, they are not ideal as yet. The kind of antibodies detected will also depend much upon the technique used, as methods selective for high avidity antibodies differentiate better between SLE and non-SLE sera (24).

Significance of DNA antibody

It is now accepted that the detection of antibodies to

dsDNA has a quite remarkable degree of specificity for SLE, despite the loss of tolerance also to other nucleic acid antigens. High binding activity of serum for dsDNA is unquestionably of diagnostic value (25) and the presence of antidsDNA antibody almost defines spontaneously developing SLE, (in contrast to drug-induced LE), although this parameter is not in the ARA criteria for the classification of SLE. The correlation of levels of high binding antibodies with clinical activity, with progress of the disease, or even with renal involvement may be disappointingly erratic (26) but together with hypocomplementemia and clinical parameters of disease activity, they are useful in guiding therapy. In cerebral lupus on the other hand, fluctuations in the levels of DNA binding antibodies do not seem to correlate with the clinical activity. In part this is contributed by the poor criteria in defining cerebral lupus. Laboratory markers of active neurologic involvement in SLE have included the measurement of cerebrospinal fluid (CSF) complement C4 (27, 28), CSF DNA-antiDNA complexes and DNA antibodies (29) and serum brain-reactive lymphocytotoxic antibodies (30). More recently, CSF cyclic GMP was found to correlate with active neurologic disease (31), but this was not specific for SLE, occurring also with other pathologic states. Thus elevated CSF cyclic GMP is useful only as a marker of active central nervous system dysfunction regardless of its aetiology.

Miniter et al (32) following up SLE patients longitudinally over three years, found that only slightly more than half of the episodes of active disease were associated with low levels of total hemolytic complement (CH50) and high dsDNA binding, and there was a significant occurrence of high DNA binding or low CH50 with inactive disease. In contrast, they found no dsDNA antibody (as measured by complement fixation using ^{14}C dsDNA from E. coli and ^{14}C poly dAT) in the absence of clinical disease. Further most episodes of central nervous disease occurred without depressed CH50 or high dsDNA binding. Thus it would appear that besides the quantity of dsDNA antibodies, their varied qualitative characteristics have to be considered.

Avidity of dsDNA antibody

Gershwin and Steinberg (33) investigated some of these qualitative characteristics and found that patients with lupus nephritis had either precipitating antibodies to DNA or a mixture of precipitating and nonprecipitating antibodies whereas those without nephritis had only nonprecipitating antibodies to DNA. Furthermore, the avidity for DNA was greatest in sera from patients with nephritis. Since then conflicting results on the avidity of antiDNA antibody in lupus nephritis have appeared (34-37). In mice, non-precipitating and low avidity antibodies are important in the development of glomerulonephritis (38). In rabbits, those producing low avidity antibodies developed membranous glomerulonephritis while those with high avidity produced membranoproliferative glomerulonephritis (39). Human studies by Asano and Nakamoto (37) showed that high avidity antiDNA antibodies localised in immune complexes in the mesangial and subendothelial positions while those with low avidity antibodies were associated with subepithelial deposits. Contrary results were reported by others (33, 35). Winfield et al (34) however postulated that high avidity antidsDNA antibodies are primarily responsible for the renal injury induced by DNA-anti-dsDNA immune complexes and are removed quickly from the circulation after the combination with dsDNA, leaving the lower avidity antibodies in the serum. Critical to this problem of avidity measurements is the many different methods used:- dissociation technique (38), association constants using serial dilutions of antibody (Scatchard's technique) (35, 36), the slopes of plots of % bound DNA against serial antibody dilutions (33) and Sips plots of 1/bound and 1/free antigen using different antigen dilutions (34), and they may not all be measuring the same quality of these antibodies. All the studies, except one, have concentrated on the sera of patients rather than the renal eluates.

Complement-fixing ability of dsDNA antibody

Beaulieu and co-workers (40) using the kinetoplast of Crithidiae luciliae to study the antibodies to dsDNA found that complement fixing activity varied independently of antibody content in the whole serum and in IgG fractions. Thus antibodies to dsDNA constitute a heterogenous population in terms of ability to activate complement. The importance of these differences lies in the concept that antibodies to DNA produced in patients with active nephritis may be of a different quality to those produced in patients without active nephritis, and these same workers found that this correlation of complement fixing with activity of lupus nephritis appears related to qualititative rather than solely quantitative differences. Earlier work by Sontheimer and Gilliam (41) showed that nephritis patients usually had dsDNA antibodies in all three major Ig classes and more frequently had the IgG dsDNA antibody subclasses that fix complement, that is, IgG1 and IgG3. IgM, IgG1 and IgG3 antibodies can activate complement via the classical pathway. However, IgA cannot, but does so by the alternate pathway. But, a more recent report (32), provides evidence that differences in complement-fixing activity are not due to class or subclass composition of the dsDNA population of antibodies as previously suggested (41-43). These same workers also found complement fixing dsDNA antibodies associated mainly with episodes of renal disease, whereas all types of disease activity except those involving predominantly the central nervous system, showed some correlation with the combined parameters of low CH50 and high dsDNA binding.

Immunoglobulin class of dsDNA antibody

Despite the uncertain correlation of complement fixing ability with the immunoglobulin class of dsDNA antibodies (as discussed above), there appears to be some correlation of the immunoglobulin class with disease activity and severity. The mechanisms of tissue injury for these relationships is at present unclear. Patients with predominantly IgM dsDNA antibodies in the serum had less active disease, mild or

no renal involvement, and longer survival than those with predominantly IgG dsDNA antibodies in the serum. Renal biopsies in patients with predominantly IgM dsDNA antibodies in the serum showed relatively benign histologic changes in the kidney while in contrast, those with predominantly IgG dsDNA antibodies showed more severe histologic changes. It was therefore suggested that the immunoglobulin class of dsDNA antibody was a critical factor in determining whether diffuse proliferative glomerulonephritis developed (44), with IgG DNA complexes producing more severe renal injury than IgM DNA complexes (45). Talal et al (46) and others (21) also correlated IgG dsDNA antibodies with severe renal lupus. However, a most recent paper by Clough and Valenzuela (47) showed that the group of patients with diffuse proliferative glomerulonephritis as contrasted with another group with focal proliferative glomerulonephritis, had a higher IgM to IgG ratio of dsDNA antibody, with both groups having almost identical total antidsDNA antibody levels. The IgA dsDNA antibody levels were not significantly different.

In the skin of lupus patients, there is a good correlation between the immunoglobulin classes of dsDNA antibodies present in the serum and that stained at the dermoepidermal junction (44). Also the skin lesions are similar to the renal lesions in SLE in that deposits containing immunoglobulin and complement are present (48). The deposits in uninvolved skin in SLE correlate with depressed CH50, high titres of dsDNA antibody and renal disease (49). A recent study by Sontheimer and Gilliam (50) demonstrated that the intensity of band fluorescence in clinically normal skin of SLE patients is directly related to the anti-dsDNA antibody titre as determined by the Crithidiae luciliae indirect immunofluorescence assay. Furthermore, the presence of IgG alone or in combination with other immunoglobulins in a positive lupus band test from clinically normal non-sun-exposed skin correlated with more marked disease activity, than the presence of IgM alone which indicated mild clinical disease (44, 51).

Immune complexes in SLE

Immune complexes are macromolecules formed as a result of combination of antigen and antibody. These macromolecules may vary greatly in size and structure depending on the size of the antigen, the class and avidity of the antibody, the lattice-work structure which itself is dependent on the valency of the antigen and antibody and the configuration of the reacting molecules. Immune complexes may be physiological in clearing antigens, this rapid elimination being dependent on phagocytosis by mononuclear and polymorphonuclear phagocytic cells. Phagocytosed antigen is rapidly catabolised. In other circumstances, immune complexes may persist and it has been suggested that low avidity antibodies may do this (52). Immune complexes may also exert important effects on cellular immune responses.

Factors which favour localisation of circulating immune complexes in vessel walls include hydrodynamic forces, filtering pressure across the vascular basement membrane, the state of vascular permeability, size, lattice structure of immune complexes and their avidity for specific tissues (53). In renal glomerular vessels, size of the macromolecules has been thought important in determining the subendothelial, intra or extra-membranous location of the immune complexes (54).

The pathogenic significance of immune complexes in SLE is widely accepted as there is evidence for the presence of circulating DNA, antibodies to DNA, immune complexes composed of DNA and antiDNA antibodies (55, 56), activation of the complement cascade leading to hypocomplementemia and deposition of DNA-antiDNA antibody and complement components in tissues, especially the glomeruli (57). There is an increased prevalence of immune complexes in SLE and assays for circulating immune complexes may have a value in the assessment of clinical activity of disease (58, 59-63). To be more specific, circulating DNA-antiDNA complexes have been demonstrated in SLE patients' sera by some workers (56, 64) but others (65) have failed to detect them. In SLE, immune complexes of molecular weight exceeding 2500,000 spared the kidneys altogether (66). Thus high levels of circulating immune complexes in SLE without analysing for DNA-antiDNA in them cannot implicate this system as other antigen-antibody systems may be involved instead (62, 67). Even when circulating DNA-antiDNA immune complexes are found, this does not indicate whether or not these circulating immune complexes deposit in tissues or are cleared by the reticuloendothelial system. There is evidence for the local formation of the immune complexes found in pathological lesions (65, 68). Izui and co-workers showed that in vitro DNA tends to bind spontaneously to isolated glomerular basement membrane and to collagen and that injected radiolabelled DNA showed a very big degree of binding in the kidneys of mice given intravenous lipopolysaccharides (LPS) (65, 69, 70). Immune complexes containing DNA in kidneys of such LPS-treated mice were also shown. Thus an alternative hypothesis to SLE lesions being the result of DNA-antiDNA immune complexes formed in the blood stream and then being deposited in capillary beds is the postulate that DNA released from cells, bacteria or viruses is bound by collagen or basement membrane; simultaneously present circulating antiDNA antibodies would thus be immunoadsorbed to tissue-bound DNA and give rise to in-situ DNA-antiDNA complexes and resultant inflammation.

Miniter et al (32) reported the association of rash with depressed CH50 alone when rash was the predominant manifestation of clinical activity. Also there were patients with rash who had low CH50 and high dsDNA titres but in none of these cases was the dsDNA complement fixing and most interestingly, although immunoglobulin and complement deposits are present in similarity to the renal lesions, there is no direct evidence that DNA-antiDNA complexes are present in the skin lesions (48, 71).

Besides the presence of circulating dsDNA antibodies and immune complexes composed of DNA-antiDNA, circulating dsDNA unbound to its antibody has been detected (72) and found to occur specifically

in patients with SLE who have vasculitis and central nervous system involvement. Swaak and colleagues (73) showed in a longitudinal study that when dsDNA antibody titres remained high (they used circular DNA in the Farr assay), no exacerbations were observed but a sharp drop in antidsDNA, usually preceded by a rise, was related to a serious exacerbation. It is accepted that in some patients, antidsDNA antibody per se is not very harmful as these patients have had high levels of them without major disease activity. The sudden fall in anti-dsDNA antibody might represent complex formation with DNA and thus fit in well with the theory involving DNA-antiDNA in the pathogenesis of SLE. The presence, then, of free circulating dsDNA might imply that all the antidsDNA antibody has been complexed and deposited already.

Reticuloendothelial system dysfunction

In a dynamic situation where free DNA as antigen, either circulating or tissue-bound, and circulating dsDNA antibody on the one hand, combine to give immune complexes on the other, the equilibrium or otherwise is dependent to a large extent on the clearance of immune complexes from the circulation. Besides capillary bed deposition or localisation to tissues where the DNA is bound, the reticuloendothelial system must be implicated. In SLE, abnormal clearances by this system correlated with immune complexes titres in the serum and disease activity. It is suggested that the defect in Fc-receptor function of the reticuloendothelial system (RES) may lead to the prolonged circulation of immune complexes thereby contributing to tissue deposition and damage (74). Blockade or saturation of the RES, which retards clearance of immune complexes markedly enhances renal deposition of complexes in laboratory animals (75). Preformed immune complexes, especially those of a larger lattice size, injected into the circulation of laboratory animals are removed rapidly by the RES (76). Efficient clearance of immune complexes will therefore prevent their tissue deposition. The importance of immunoglobulin class of antibody in determining which effector mechanisms an immune complex can activate and in clearance of such complexes by the RES has been recognised (77). It is possible that IgG binding to DNA produces immune complexes of a size that can be tissue deposited but cannot be readily cleared by the RES. Mannik and Arend (78) reported that immune complexes containing two or less IgG antibodies per complex circulate in the blood of humans for many hours whereas IgM complexes are removed rapidly by the RES. Complexes larger than Ag2Ab2 containing IgG1 or IgG3 antibody should be rapidly removed by the RES because of the presence of Fc-receptors for these antibodies on macrophages.

Size of the immune complexes is another important factor. Immune complexes of large size (35S) are formed primarily during periods of antibody excess. Large complexes of dsDNA and anti-dsDNA antibody formed during periods of high dsDNA antibody titre may localise primarily in peripheral tissues and the RES (79). These large complexes are less likely to reach the kidneys. Small complexes (11 - 19S) do not localise in peripheral tissues but pass through the vasculature and become trapped, e.g. in glomeruli. Since small immune complexes are formed in times of antigen excess, they cannot be present if unbound antibody is present in the sera in significant amounts. Thus the failure of the dsDNA antibody titre to correlate with certain SLE manifestations suggests that small immune complexes may play a primary pathogenic role.

ssDNA antibodies

Antibodies to ssDNA occur in SLE but also in a host of other rheumatic diseases. These antibodies often give a falsely high value to tests designed to detect only dsDNA antibodies because of the impurity of double-strandedness of the DNA antigen. Heinzerling and co-workers (21) using a solid phase radioimmunoassay to quantitate ssDNA antibodies found that the presence of IgG antibodies to ssDNA was associated with renal involvement while patients with IgM antibodies to ssDNA alone had more benign types of SLE with little renal involvement. They also found that ssDNA as the antigen provided greater binding efficiency than did dsDNA suggesting that most of the antibodies assayed for in active SLE patients reacted with purine and pyrimidine bases or a combination of these bases rather than polydeoxyribose phosphate "backbone". Other studies (80) showed that ssDNA blocked binding of the antibodies to dsDNA better than dsDNA itself. Picazo and Tan (81) suggest that antiDNA antibody in many SLE patients may be strongest to repeating adenine and thymidine nucleotides in dsDNA, illustrating that nucleotide bases are the antigenic sites for most DNA antibodies.

Interference by anti-extractable nuclear antigen antibodies?

In 1972, Sharp and his colleagues described an overlap syndrome of SLE, generalised scleroderma and polymyositis-dermatomyositis, and named it mixed connective tissue disease (82). The underlying feature of this concept was the presence of antibodies to a saline extractable nuclear antigen (ENA) that was RNAase sensitive. ENA has two distinct moieties - soluble ribonucleoprotein (RNP) and a glycoprotein termed "Sm antigen" (83). RNAase sensitive ENA is synonymous with RNP: RNP antibodies are a sine qua non for the diagnosis of mixed connective tissue disease, although they are also found in a small proportion of patients with classical scleroderma and SLE (84-87). Notman et al have shown that Sm antibodies are restricted to patients with SLE (88) and Reichlin has suggested that the mixed connective tissue disease syndrome is most compatible with SLE favourably modified by the presence of RNP antibodies (87, 89, 90).

Powers et al reported 16 SLE patients who had circulating Sm antibodies and suggested that this serologic pattern might be indicative of a more benign form of SLE with non-progressive nephritis (91). 135 SLE patients with DNA (detected by C. luciliae) and/or Sm antibodies were compared to identify a specific

lupus subset. In their study, Winn et al (92) found Raynaud's phenomenon more frequently in patients with Sm antibodies while serious central nervous system disease was over three times as common in patients with DNA antibodies. They therefore felt that the Sm antibody system may identify a subset of SLE patients with milder central nervous system and renal disease. The postulated mechanism is that binding of Sm to DNA-antiDNA complexes alters the size, configuration or complement affinity of these immune complexes thus interfering with their recognition by receptors in the glomerular basement membrane (93) and also in the choroid plexus (92, 94, 95). Basis for this postulate stems from two observations: one, that Sm has a strong binding for singlestranded DNA and can inhibit a DNA-antiDNA reaction in vitro (96), and two, that injections of Sm antigen can reduce the severity of nephritis of NZB/NZW mice (97).

A low incidence of renal disease has been noted in SLE patients with RNP antibodies (98, 99) and ENA has been shown to interact with DNA, possibly inhibiting DNA-antiDNA immune complexes (100). It thus appears that SLE patients with antibodies to ENA in addition to their usual spectrum of autoantibodies, have a more benign form of disease.

Immunoregulatory dysfunction: aberration in the switchover mechanism?

From the evidence so far, it seems that the IgG class of antibody to dsDNA and also ssDNA (21), is more closely related to disease severity than the IgM class of autoantibodies. In animal models, many types of autoantibodies as they progressively appear, change in the class of immunoglobulin produced from 19S (IgM) to 7S (IgG). Further this switch to IgG production occurs earlier and with more severe kidney disease in the female than in the male. Going a step further, Talal et al (101, 102) found the presence of only 19S antibody to RNA in asymptomatic relatives while 7S antibody to DNA and RNA was found to correlate with active SLE in patients. Other workers (41, 44) reported the predominance of IgG anti-DNA antibody in active SLE with nephritis as opposed to SLE without nephritis. IgM antibody was found mainly in relatively mild disease. In contrast, Clough and Valenzuela (47) found the reverse with more IgM antiDNA in diffuse proliferative glomerulonephritis, the lesion with the worst prognosis in SLE, than in focal proliferative glomerulonephritis.

Besides DNA antibodies, these differences in immunoglobulin class are found also with lymphocytotoxic antibodies. Cold reactive lymphocytotoxic antibodies (LCTA) have been described in a variety of diseases including acute and chronic viral and bacterial infections, several types of malignancy, and a majority of the diseases currently associated with autoimmunity. In patients, LCTA have been shown to vary directly with certain symptoms and signs of SLE. Active arthritis, serositis, skin rash, lymphopenia, and hypocomplementemia are all associated with the presence of these antibodies (103-105). Messner and De Horatius (106) found LCTA in 80% of SLE patients and 40-60% of their asymptomatic relatives occurring equally in consanguineous and non-sanguineous relatives but are more common with close proband contact. These antibodies are primarily IgM in relatives but are IgM and IgG in SLE patients.

These data suggest that SLE patients have a defect in the controls that normally restrict antibodies to the IgM class, allowing the switch to IgG antibodies that have a more pathogenic potential. After successful treatment, the antibody type progressively reverts from IgG to IgM (107). The switch from IgM to IgG production often involves the action of lymphocyte T cells.

CONCLUSION

The clinical heterogeneity of SLE may be due to variations in the genetic and environmental factors that determine the appearance of overt disease and the organ systems involved. Whether spontaneous SLE is a disease of cellular immunological dysfunction (i.e. loss of lymphocyte suppressor T cell function) with resultant lymphocyte B cell hyperactivity, or a disease of primarily B cell hyper-reactivity, remains unresolved. Environmental factors may elicit the development of autoantibodies that in the absence of a genetic predisposition may cause no disease (i.e. relatives of SLE probands with LCTA). However in the presence of genetic factors, the environmental determinants may contribute to the onset of SLE and determine its manifestations and course of the patient's disease. Genetic predisposition and hormonal factors may trigger development of overt and/or severe disease by causing antibodies to switch from IgM to IgG class. The intensity of this antibody response, the load of free dsDNA as antigen in the circulation or at tissue sites, the interaction between them (depending on avidity and complement fixing ability among other things), and the capacity of the RES to eliminate pathogenic immune complexes must all come into the picture. Perhaps in the same individual, the above stages may vary with each step of the clinical course. That dsDNA has been discussed reflects the specificity of this auto-antibody-antigen system in spontaneous SLE, which is in marked contrast to its absence or low titre in drug-induced lupus erythematosus. The latter rarely causes cerebral or renal lupus. Much remains to be investigated in SLE, despite the intense research of past years that have culminated in the present concepts as discussed in this article.

REFERENCES

1. Ceppellini R, Poll E, Celado F: A DNA-reacting factor in serum of a patient with lupus erythematosus diffusus. Proc Soc Exp Biol Med 1957; 96: 572-574.
2. Deicher HRG, Holman HR, Kunkel HG: The precipitin reaction between DNA and a serum factor in systemic lupus erythematosus. J Exp Med 1959; 109: 97-114.
3. Rothfield NF, Stollar BD: 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 1967; 46: 1785-1794.
4. Schur PH, Sandson J: Immunological factors in

systemic lupus erythematosus. N Engl J Med 1968; 278: 533-538.
5. Hughes GRV, Cohen AS, Christian CL: Anti-DNA activity in systemic lupus erythematosus: A diagnostic and therapeutic guide. Ann Rheum Dis 1971; 30: 259-264.
6. Locker JD, Medof ME, Bennett RM, Sukhupunyaraksa S: Characterisation of DNA used to assay sera for anti-DNA antibodies; determination of the specificities of anti-DNA antibodies in SLE and non-SLE rheumatic disease states. J Immunol 1977; 118: 694-701.
7. Arana R, Seligmann M: Antibodies to native and denatured deoxyribonucleic acid in systemic lupus erythematosus. J Clin Invest 1967; 46: 1867-1882.
8. Stollar BD: in Report of Symposium on "Antinuclear antibodies and their clinical significance". Ann Rheum Dis 1976; 35: 466-472.
9. Brentjens J, Ossi E, Albini B et al: Disseminated immune deposits in lupus erythematosus. Arthritis Rheum 1977; 20: 962-968.
10. Koeffler D, Schur PH, Kunkel HG: Immunological studies concerning the nephritis of systemic lupus erythematosus. J Exp Med 1967; 126: 607-623.
11. Tan EM, Schur PH, Carr RI, Kunkel HG: Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. J Clin Invest 1966; 45: 1732-1740.
12. Koeffler D, Carr R, Agnello V, Thoburn R, Kunkel HG: Antibodies to polynucleotides in human sera: antigenic specificity and relation to disease. J Exp Med 1971; 134: 294-312.
13. Koeffler D, Agnello V, Winchester R, Kunkel HG: The occurrence of singlestranded DNA in the serum of patients with systemic lupus erythematosus and other diseases. J Clin Invest 1973; 52: 198-204.
14. Andres GA, Accinni L, Beiser SM et al: Localisation of fluorescein-labeled antinucleoside antibodies in glomeruli of patients with active SLE nephritis. J Clin Invest 1970; 49: 2106-2118.
15. Aarden LA: in Report of Symposium on "Antinuclear antibodies and their clinical significance". Ann Rheum Dis 1976; 35: 466-472.
16. Aarden LA, de Groot ER, Feltkamp TEW: Immunology of DNA III. Crithidiae luciliae, a simple substrate for the determination of anti-dsDNA with the immunofluorescent technique. Ann NY Acad Sci 1975; 254: 505-515.
17. Steinman CR, Deesomchok U, Spiera H; Proceedings: Detection of antibody to native DNA (nDNA): increased specificity for active SLE by using a synthetic nDNA antigen free of contaminating single-stranded DNA (ssDNA). Arthritis Rheum 1975; 18: 284.
18. Aarden LA, Lakmaker F, Feltkamp TE: Immunology of DNA I. The influence of reaction conditions on the Farr assay as used for the detection of anti-dsDNA. J Immunol Methods 1976; 10: 27-37.
19. Vogel JC, Roberts JL, Lewis EJ: A non-anti-DNA antibody detected with the Crithidiae luciliae antiDNA assay (correspondence). N Engl J Med 1980; 303: 458-459.
20. Steinman CR, Grishman E, Spiera H, Deesomchok U: Binding of synthetic double-stranded DNA by serum from patients with SLE. Am J Med 1977; 62: 319-322.
21. Heinzerling RH, Dzinba DS, Federyszyn HM, Burnham TK: Significance of levels of specific immunoglobulins to DNA in SLE patients' sera detected by solid phase radioimmunoassay. J Invest Dermatol 1979; 72: 55-58.
22. Geisert M, Heicke B, Metzmann E, Zahn RK: Influence of molecular weight of DNA on the determination of antiDNA antibodies in SLE sera by radioimmunoassay. Nucl Acids Res 1975; 2: 521-535.
23. Aarden LA, Lakmaker F, Feltkamp TEW: Immunology of DNA II. The effect of size and structure of the antigen on the Farr assay. J Immunol Methods 1976; 10: 39-48.
24. Aarden LA: Measurement of antiDNA antibodies. Ann Rheum Dis 1977; 36 suppl: 91-95.
25. Hughes GRV: Systemic Lupus Erythematosus. In JT Scott, ed. Copeman's Textbook of the Rheumatic Diseases. Churchill Livingston, Edinburgh and London. 5th edition, 1978; 901-922.
26. Edmonds JP, Johnson GD, Ansell BM, Holborrow EJ: The value of tests for antibodies to DNA in monitoring the clinical course of SLE. Clin Exp Immunol 1975; 22: 9-15.
27. Petz LD, Sharp GC, Cooper NR, Irvin WS: Serum and cerebrospinal fluid complement and serum autoantibodies in SLE. Medicine 1971; 50: 259-275.
28. Hadler NM, Gerwin RD, Frank MM, Whitaker JN, Baker M, Decke JL: The fourth component of complement in the cerebrospinal fluid in SLE. Arthritis Rheum 1973; 16: 507-521.
29. Keefe EB, Bardana EJ, Harbeck RJ, Pirofsky B, Carr RI: Lupus meningitis: antibody to DNA and DNA-antiDNA complexes in cerebrospinal fluid. Ann Intern Med 1974; 80: 58-60.
30. Bluestein HG, Zvaifler NJ: Brain-reactive lymphcytotoxic antibodies in the serum of patients with SLE. J Clin Invest 1976; 57: 509-516.
31. Kassan SS, Kagen LJ: Central nervous system lupus erythematosus. Measurement of cerebrospinal fluid cyclic GMP and other clinical markers of disease activity. Arthritis Rheum 1979; 22: 449-457.
32. Miniter MF, Stollar BD, Agnello V: Reassessment of the clinical significance of native DNA antibodies in SLE. Arthritis Rheum 1979; 22: 959-968.
33. Gershwin ME, Steinberg AD: Qualitative characteristics of anti-DNA antibodies in lupus nephritis. Arthritis Rheum 1974; 17: 947-954.
34. Winfield JB, Faiferman I, Koffler D: Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with SLE. Association of high avidity anti-native DNA with glomerulonephritis. J Clin Invest 1977; 59: 90-96.
35. Leon SA, Green A, Ehrlich GE, Polando M, Shapiro M: Avidity of antibodies in SLE. Relation to severity of renal involvement. Arthritis Rheum 1977; 20: 23-29.
36. Tron F, Bach JF: Relationships between antibodies to native DNA and glomerulonephritis in SLE. Clin Exp Immunol 1977; 28: 426-432.
37. Asano Y, Nakamoto Y: Avidity of anti-native DNA antibody and glomerular immune complex localisation in lupus nephritis. Clin Nephrol 1978; 10: 134-139.
38. Steward MW, Katz FE, West NJ: The role of low affinity antibody in immune complex disease. The quantity of antiDNA antibodies in NZB/WF, hybrid mice. Clin Exp Immunol 1975; 21: 121-130.
39. Kuriyama T: Chronic glomerulonephritis induced by prolonged immunization in the rabbit. Lab Invest 1973; 28: 224-235.
40. Beaulieu A, Quismoro FP, Friou GJ, Vayuvegula B, Mirick G: IgG antibodies to dsDNA in SLE. Arthritis Rheum 1979; 22: 565-570.
41. Sontheimer RD, Gilliam JN: DNA antibody class, subclass and complement fixation in SLE with and without nephritis. Clin Immunol Immunopathol 1978; 10: 459-467.
42. Schur PH, Monroe M, Rothfield N: The gamma G subclass of antinuclear and antinucleic acid antibodies. Arthritis Rheum 1972; 15: 174-182.

43. Puritz EM, Yount WJ, Newell M: Immunoglobulin classes and IgG subclasses of human antinuclear antibodies. A correlation of complement fixation and the nephritis of SLE. Clin Immunol Immunopathol 1973; 2: 98-113.
44. Pennebaker JB, Gilliam JN, Ziff M: Immunoglobulin classes of DNA binding activity in serum and skin in SLE. J Clin Invest 1977; 60: 1331-1338.
45. Koeffler D, Agnello V, Carr RI, Kunkel HG: Anti-DNA antibodies and renal lesions of patients with SLE. Transplant Proc. 1969; 1: 933-938.
46. Talal N, Roubinian JR, Pillarisetty RJ, Shigemasa S: Biological significance of IgM and IgG antibodies to DNA and RNA in autoimmune disease. Am J Clin Pathol 1977; 68: 643-646.
47. Clough JD, Valenzuela R: Relationship of renal histopathology in SLE nephritis to immunoglobulin of anti-DNA. Am J Med 1980; 68: 80-85.
48. Tan EM, Kunkel HG: An immunofluorescent study of the skin lesions in SLE. Arthritis Rheum 1966; 9: 37-46.
49. Gilliam JN, Cheatum DE, Hurd ER, Stastney P, Ziff M: Immunoglobulin in clinically uninvolved skin in SLE. J Clin Invest 1974; 53: 1434-1440.
50. Sontheimer RD, Gilliam JN: A reappraisal of the relationship between subepidermal immunoglobulin deposits and DNA antibodies in SLE: a study using Crithidiae luciliae immunofluorescence antiDNA assay. J Invest Dermatol 1979; 72: 29-32.
51. Harrist TJ, Mihm MC: The specificity and clinical usefulness of the lupus band test. Arthritis Rheum 1980; 23: 479-490.
52. Soothill JF, Steward MW: The immunopathological significance of the heterogeneity of antibody affinity. Clin Exp Immunol 1971; 9: 193-199.
53. Maini RN: Circulating immune complexes: current concepts of their pathogenetic role and methods of detection. Aust NZ J Med 1978; 8 suppl: 68-76.
54. Oliver C, Essner E: Protein transport in mouse kidney utilising tyrosinase as an ultrastructural tracer. J Exp Med 1972; 136: 291-304.
55. Davis JS, Godfrey SM, Winfield JB: Direct evidence for circulating DNA/anti-DNA complexes in SLE. Arthritis Rheum 1978; 21: 17-22.
56. Harbeck RJ, Bardana EJ, Kohler PF, Carr RI: DNA: anti-DNA complexes in SLE. J Clin Invest 1973; 52: 789-795.
57. Koffler D, Agnello V, Kunkel HG: Polynucleotide immune complexes in serum and glomeruli of patients with SLE. Am J Pathol 1974; 74: 109-124.
58. Abrass CK, Nies KM, Louie JS, Border WA, Glassock RJ: Correlation and predictive accuracy of circulating immune complexes with disease activity in patients with SLE. Arthritis Rheum 1980; 23: 273-282.
59. Nydegger UE, Lambert PH, Gerber H, Miescher PA: Circulating immune complexes in the serum in systemic lupus erythematosus and in carriers of hepatitis-B antigen. J Clin Invest 1974; 54: 297-309.
60. Hay FC, Nineham LJ, Roitt IM: Routine assay for the detection of immune complexes of known immunoglobulin class using solid phase C1q. Clin Exp Immunol 1976; 24: 396-400.
61. Cano PO, Jerry LM, Sladowski JP, Osterland CK: Circulating immune complexes in systemic lupus erythematosus. Clin Exp Immunol 1977; 29: 197-204.
62. Agnello V, Koffler D, Kunkel HG: Immune complex systems in the nephritis of systemic lupus erythematosus. Kidney Int 1973; 3: 90-99.
63. Casali P, Bossus A, Carpenter NA, Lambert PH: Solid phase enzyme immunoassay or radioimmunoassay for the detection of immune complexes based on their recognition by conglutinin: conglutinin binding test: a comparative study with ^{125}I-labeled C1q binding and Raji cell RIA tests. Clin Exp Immunol 1977; 29: 342-354.
64. Bruneau C, Benveniste J: Circulating DNA: antiDNA complexes in SLE. J Clin Invest 1979; 64: 191-198.
65. Izui S, Lambert PH, Meischer PA: Failure to detect circulating DNA anti-DNA complexes by four radioimmunological methods in patients with SLE. Clin Exp Immunol 1977; 30: 384-392.
66. Levinsky RJ, Cameron JS, Soothill JF: Serum immune complexes and disease activity in lupus nephritis. Lancet 1977; 1: 564-567.
67. Agnello V, Koffler D, Eisenberg JW, Winchester RJ, Kunkel HG: C1q precipitins in the sera of patients with SLE and other hypocomplementemic states: characterisation of high and low molecular weight types. J Exp Med 1971; 134: 228s-241s.
68. Stingl G, Meingassner JG, Swelty P, Knapp N: An immunofluorescence procedure for the demonstration of antibody to native double-stranded DNA and of circulating DNA-antiDNA complexes. Clin Immunol Immunopathol 1976; 6: 131-140.
69. Izui S, Lambert Ph Fournie GJ, Turler H, Meischer PA: Features of SLE in mice injected with bacterial lipopolysaccharides. Identification of circulating DNA and renal localisation of DNA anti-DNA complexes. J Exp Med 1977; 145: 1115-1130.
70. Izui S, Lambert PH, Meischer PA: 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 SLE. J Exp Med 1976; 144: 428-443.
71. Van Joost TH: An attempt to identify the nature of immunoglobulin-complement deposits in the skin in lupus erythematosus. Brit J Dermatol 1973; 89: 15-20.
72. Steinman CR. Circulating DNA in SLE. Am J Med 1979; 67: 429-435.
73. Swaak AJG, Aarden LA, Statius van Eps LW, Feltkamp TEW: AntidsDNA and complement profiles as prognostic guides in SLE. Arthritis Rheum 1979; 22: 226-235.
74. Frank MM, Hamburger MI, Lawley TJ, Kimberly RP, Plotz PH: Defective reticuloendothelial system Fc-receptor function in SLE. N Engl J Med 1979; 300: 518-523.
75. Haakenstad AO, Mannik M: Saturation of the reticuloendothelial system with soluble immune complexes. J Immunol 1974; 112: 1939-1948.
76. Mannik M, Arend WP, Hall AP, Gilliland BC: Studies on the antigen-antibody complexes. I. Elimination of soluble complexes from rabbit circulation. J Exp Med 1971; 133: 713-739.
77. Mannik M, Haakenstad AO, Arend WP: Progress in immunology II Vol 5; eds. Brent L and Holborrow J; North Holland Publishing Company, Amsterdam, 1974, p 51.
78. Mannik M, Arend WP: Fate of preformed immune complexes in rabbits and rhesus monkeys. J Exp Med 1971; 138: 19s-31s.
79. Henson PM: Immune complex diseases: cellular mediators and the pathogenesis of the inflammatory tissue injury produced by immune complexes. In Glynn LE and Schlumberger HD eds. Experimental models of chronic inflammatory diseases. New York, Springer Verlag, 1977; 94-106.
80. Levine L, Stollar BD: Nucleic acid immune systems. Progr Allergy 1968; 12: 161-191.
81. Picazo JT, Tan EM: Specificities of antibodies to native DNA. Scand J Rheumatol 1975; 11 suppl: 35-41.
82. Sharp GC, Irwin WS, Tan EM, Holman H: Mixed connective tissue disease - an apparently distinct

rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). Am J Med 1972; 52: 145-159.
83. Mattioli M, Reichlin M: Physical association of two nuclear antigens and the mutual occurrence of their antibodies: The relationship of the Sm and RNA protein (Mo) system in SLE sera. J Immunol 1977; 110: 1318-1324.
84. Parker MD: Ribonucleoprotein antibodies: Frequency and clinical significance in SLE, scleroderma and mixed connective tissue disease. J Lab Clin Med 1973; 82: 769-775.
85. Leibfarth JH, Persellin RH: Characteristics of patients with serum antibodies to extractable nuclear antigens. Arthritis Rheum 1976; 19: 851-856.
86. Sharp GC, Irwin WS, May CM et al: Association of antibodies to ribonucleoprotein and Sm antigens with MCTD, SLE and other rheumatic diseases. N Engl J Med 1976; 295: 1149-1154.
87. Maddison PJ, Mogovero H, Reichlin M: Patterns of clinical disease associated with antibodies to nuclear ribonucleoprotein. J Rheumatol 1978; 5: 407-411.
88. Notman DD, Kurata N, Tan EM: Profile of antinuclear antibodies in systemic rheumatic disease. Ann Intern Med 1975; 83: 464-469.
89. Reichlin M, Mattioli M: Antigens and antibodies characteristic of SLE. Bull Rheum Dis 1974; 24: 756-760.
90. Reichlin M: MCTD, In Hughes GRV, ed. Modern Topics in Rheumatology. London, Heinemann 1976; 162-166.
91. Powers R, Akizuki M, Boehm-Truitt M, Daly V, Holman HR: Substantial purification of the Sm antigen and association of high titre antibody to Sm with a clinical subset of lupus erythematosus (SLE) (abstr). Arthritis Rheum. 1977; 20: 131.
92. Winn DM, Wolfe JF, Lindberg DA, Fristoe FH, Kingsland L, Sharp GC: Identification of a clinical subset of SLE by antibodies to the Sm antigen. Arthritis Rheum 1979; 22: 1334-1337.
93. Gelfand MC, Shin ML, Nagle RB, Green I, Frank MM: The glomerular complement receptor in immunologically mediated renal glomerular injury. N Engl J Med 1976; 295: 10-14.
94. Atkins CJ, Kondon JJ Jr, Quismorio FP, Friou GJ: The choroid plexus in SLE. Ann Intern Med 1972; 76: 67-72.
95. Peress NS, Miller F, Palu W: The choroid plexus in passive serum sickness. J Neuropathol Exp Neurol 1977; 36: 561-566.
96. Reyes PA, Tan EM: DNA binding property of Sm nuclear antigen. J Exp Med 1977; 145: 749-754.
97. Morris AD, Littleton C, Corman LC, Esterly J, Sharp GC: Extractable nuclear antigen effect on the DNA-antiDNA reaction and NZB/NZW mouse nephritis. J Clin Invest 1975; 55: 903-907.
98. Reichlin M, Mattioli M: Correlation of a precipitin reaction to RNA protein antigen and a low prevalence of nephritis in patients with SLE. N Engl J Med 1972; 286: 908-911.
99. Gaudreau A, Amar B, Kahn MF, Ryckewaert A, Sany J, Peltier AP: Clinical significance of antibodies to soluble extractable nuclear antigens (anti-ENA). Ann Rheum Dis 1978; 37: 321-327.
100. Hamburger M, Friedlander L, Borland P: Interactions of extractable nuclear antigen (ENA) and double stranded DNA. Arthritis Rheum 1974; 17: 469-475.
101. Talal N, Pillarisetty RJ, De Horatius RJ, Messner RP: Immunologic regulation of spontaneous antibodies to DNA and RNA. I. Significance of IgM and IgG antibodies in SLE patients and asymptomatic relatives. Clin Exp Immunol 1976; 25: 377-382.
102. De Horatius RJ, Pillarisetty RJ, Messner RP, Talal N: Antinucleic acid antibodies in SLE patients and their families - incidence and correlation with lymphocytotoxic antibodies. J Clin Invest 1975; 56: 1149-1154.
103. Stastny P, Ziff M: Direct lysis of lymphocytes by complement in patients with SLE. Arthritis Rheum 1971; 14: 733-736.
104. Butler WT, Sharp JT, Rosen RD, Lidsky MD, Mittal KK, Gard DA: Relationship of the clinical course of SLE to the presence of circulating lymphocytotoxic antibodies. Arthritis Rheum 1972; 15: 231-238.
105. Winfield JB, Winchester RJ, Kunkel HG: Association of cold-reactive anti-lymphocytotoxic antibodies with lymphopenia in SLE. Arthritis Rheum 1975; 18: 587-594.
106. Messner RP, De Horatius RJ: Epidemiology of anti-lymphocytotoxic antibodies in SLE. Arthritis Rheum 1978; 21: 167s-170s.
107. Talal N: in Report of Symposium on "Antinuclear antibodies and their clinical significance". Ann Rheum Dis 1976; 35: 466-472.