Clin. exp. Immunol. (1981) **44**, 1–10.

REVIEW

Current perspectives on serological reactions in SLE patients

M. REICHLIN *Departments of Medicine and Biochemistry, State University of New York at Buffalo, and Veterans Administration Medical Center, Buffalo, New York*

(Accepted for publication 17 October 1980)

INTRODUCTION

Research efforts directed toward understanding the serology of systemic lupus erythematosus patients have manifest distinct stages of development. Identification of individual antigen–antibody reactions with detailed characterization of the autoimmune responses of these patients has been a major achievement marking the period beginning with the discovery of the LE phenomenon by Hargraves, Richmond & Norton in 1948, and ending roughly in the early 1970s. In that interval, antibodies to two families of antigens were characterized: those that reacted with chemically well-defined macromolecules and a second group defined by precipitin reactions in agar gels and classified by identity reactions with selected monospecific prototype sera.

In the first antigenic group are single- and double-stranded DNA, single- and double-stranded RNA, and ribosomal constituents. In the second group are antigens which have been identified by their precipitin reactions in agar gel and are designated largely by the first two letters of the patient from whom the prototype serum originated. These include the antigens Sm (Tan & Kunkel, 1966), Mo (nRNP) (Mattioli & Reichlin, 1971); Ro (Clark, Reichlin & Tomasi, 1968), La (cytRNP) (Mattioli & Reichlin, 1974), PCNA (Miyachi, Fritzler & Tan, 1978), and Ma (Winn *et al.*, 1979a). The latter two antigens occur in relatively small numbers of SLE patients and will be described briefly to emphasize the points of interest generated by their description. During the early phases of the recognition of all these reactions, intensive contemporary clinical study established the clinical specificity and possible usefulness of these reactions. Such laboratory–clinical correlation studies emphasize the potential utility of these reactions in clinical diagnosis and in providing information on risk of specific organ involvement as well as prognosis.

This review will summarize some correlations that exist between specific antibodies and clinical diagnosis and course but will emphasize some of the newer insights into the overall immunobiological significance of these reactions that have resulted from biochemical, immunochemical, immunogenetic and molecular biological studies of these antigens and the production of their associated antibodies.

This overview will be divided into two parts corresponding to the two classes of antigens to be discussed: DNA-containing antigens and non-DNA-containing antigens.

DNA-CONTAINING ANTIGENS

Antibodies to DNA are presently the group of antibodies to a single antigen with the widest clinical applicability and among these antibodies are those with the highest specificity for the diagnosis of SLE. There are two large categories of specificities that have clinical relevance and they correspond

Correspondence: Morris Reichlin, MD, Professor of Medicine and Biochemistry, Veterans Administration Medical Center, 3495 Bailey Avenue, Buffalo, New York 14215, USA.

0099-9104/81/0400-0001$02.00

to the two macromolecular forms of DNA. These are native (*n* or double-stranded) and denatured (single-stranded or *ss*) DNA. Antibodies to nDNA are directed to some aspect of the double-helical conformation of nDNA, the exact structure as yet being undetermined. Such antibodies do not bind to the free purine and pyrimidine bases but paradoxically can be absorbed out with denatured DNA. In an investigation of this problem it has been shown that secondary structure in denatured DNA is responsible for its reaction with anti-native DNA antibodies in SLE sera (Stollar & Papalian, 1980). Studies by the latter authors demonstrated that S_1 nuclease-resistant and heat-susceptible cores of double-helical structure in denatured DNA seemed to be the source of reactivity for antibodies in SLE sera with specificity for nDNA. In a related study it was determined that amongst antibodies to nDNA in SLE sera, great heterogeneity existed in binding specificity which could be characterized by the pattern of reactivity exhibited by such antibodies with native DNA fragments varying in size from 20 to 1,200 base pairs (Papalian *et al.*, 1980). These studies support the long-held contention that specific antibodies to nDNA exist and they throw light on the previously puzzling observation that denatured DNA could absorb out such antibodies to nDNA (Stollar, 1973).

Antibodies to nDNA are highly specific for the clinical diagnosis of SLE although a rare report of the occurrence of such antibodies in rheumatoid arthritis patients does exist. These antibodies occur maximally in 70 to 75% of clinically recognizable SLE patients when they are clinically active and untreated. Since these antibodies to nDNA frequently fluctuate with disease activity and usually disappear rapidly with immunosuppressive therapy, their occurrence is very low in SLE patients who experience either a spontaneous or a steroid-induced remission. Demonstration of these antibodies requires impeccably native DNA in the assay procedure. An acceptable parameter to assess single-stranded regions in the test nDNA utilized for assay is a positive reaction with rabbit antibodies prepared against the purine or pyrimidine bases. Failure of reactivity with such antibodies is taken as strong evidence against the presence of single-stranded regions in the nDNA used for assays. The kinds of antigen preparations thought to be free of single-stranded regions include polydeoxyadenylatethymidylate (poly dAT), Crithidia kinetoplast DNA, PM_2 DNA, KB or *E. coli* nDNA treated with S_1 nuclease, and KB or *E. coli* nDNA suitably purified on benzoylated DEAE columns. The requirement of nDNA free of single-stranded regions for anti-DNA detection was recently shown in a study in which apparent reactivity of human sera for nDNA from patients with diseases other than SLE was due to antibody to single-stranded regions in the putative nDNA preparations (Locker *et al.*, 1977). A test for anti-native DNA which has gained great ascendancy in recent years is the immunofluorescent test utilizing *Crithidia luciliae* as substrate. This trypanosome contains within its kinetoplast circular DNA which is double-stranded by all physicochemical and immunochemical criteria (Aarden, deGroot & Feltkamp, 1975).

Antibodies to nDNA not only have considerable diagnostic specificity but have also been shown to be closely related to disease activity (Koffler *et al.*, 1971a; Schur & Sandson, 1968). There is considerable evidence pointing to an important pathogenetic role for nDNA–anti-nDNA immune complexes and particularly in lupus nephritis (Tan, Schur & Carr, 1966; Koffler, Schur & Kunkel, 1967; Cochrane & Koffler, 1973).

Antibodies to ssDNA probably play a major role in the immunopathogenesis of SLE. The relevance of anti-ssDNA in SLE is emphasized not only by the observation that the specificity of antibodies to DNA is most commonly to ssDNA (Koffler *et al.*, 1971b; Samaha & Irvin, 1975), but also by the identification of ssDNA in sera from lupus patients (Barnett, 1968; Koffler *et al.*, 1973), and the demonstration of the alternating appearance of antigen and antibody in the circulation, in cryoprecipitates (Winfield, Koffler & Kunkel, 1975), in renal lesions (Andres *et al.*, 1971) and in renal eluates (Koffler *et al.*, 1971a). In addition, in acid elution studies of nephritic kidneys from SLE patients such antibodies to ssDNA are invariably found in higher titres than antibodies to nDNA and about 20% of the time are found when no antibodies are present which bind nDNA (Koffler, Agnello & Kunkel, 1974).

A question that has recently been reinvestigated is why some SLE patients with substantial antibody titres to nDNA fail to develop nephritis. Two recent studies point to the absence of complement-fixing ability of the anti-native DNA in patients without apparent clinical evidence of nephritis (Miniter, Stollar & Agnello, 1979; Beaulieu *et al.*, 1979). By inference it is reasoned that the

complement-fixing properties of anti-DNA are necessary for the development of the inflammatory process in lupus nephritis.

These antibodies are, therefore, at least as important as anti-nDNA in immunopathogenesis and in some patients constitute the major demonstrable immune complex system. Unfortunately, antibodies to ssDNA are found in a proportion of patients with other connective tissue diseases, albeit in lower titres. Antibodies to ssDNA have been found in about one-third of rheumatoid arthritis patients, in chronic active hepatitis, in drug-induced SLE and in scleroderma, particularly in the active phase of the variant known as the linear localized form. Thus while antibodies to ssDNA are of both diagnostic and immunopathogenetic importance, their incomplete specificity must be recognized if their determination is to be useful without being misleading.

NON-DNA-CONTAINING ANTIGENS

Anderson *et al.* (1961) first described the presence of precipitating antibodies to antigens in crude extracts in the sera of patients with Sjögren's syndrome and since then the gel diffusion method of Ouchterlony has been utilized to characterize several such precipitating antibody systems in the sera of SLE patients. Although some laboratories have used passive haemagglutination as a method to detect and quantitate antibodies to the nuclear antigens nRNP and Sm, immunodiffusion is the only technique which can confirm immunological identity between different sera because of the heterogeneous nature of the available antigen preparations. All of the antigens to be discussed behave as soluble materials and are prepared by ultracentrifugation of tissue extracts and behave as proteins or RNA protein conjugates in their susceptibility to proteolytic and nucleolytic enzymes.

Patients with SLE develop autoantibodies to four of these soluble antigens with any great frequency, namely Sm, nuclear ribonucleoprotein (nRNP), Ro and La. Cellular localization of these antigens has been recognized by (1) cell fractionation in sucrose solutions, (2) immunofluorescence with monospecific serum, or (3) most rigorously with immunofluorescence with specifically purified antibody separated from washed immune precipitates prepared by mixing antigen with monospecific serum at equivalence.

Antibodies to the Sm and nRNP antigens

There is agreement that both Sm and nRNP antigens are nuclear in origin and as Sm is partially trypsin-sensitive, acidic and RNase-resistant, it has been designated a nuclear non-histone protein. Biochemical analysis of the composition of purified Sm antigen has not yet been reported although considerable progress has been made in its purification (Lieu & Tan, 1979). The nRNP antigen has been purified by immunoaffinity chromatography by two groups and both studies show the presence of RNA and several protein subunits in the isolated antigen in agreement with the antigen's sensitivity to both RNase and trypsin. The nRNP protein antigen recovered by Douvas *et al.* (1979) was eluted from the affinity column with 0·1 N HCl and seems to be antigenically incomplete or partially degraded since it does not give a reaction of complete identity with crude extract in gel precipitation reactions with prototype serum. The nRNA protein antigen recovered by Takano, Agris & Sharp (1980) was eluted by 3 M KSCN from the affinity column and reacts identically to crude extract in precipitin reactions with prototype serum monospecific for anti-nRNP. An interesting and provocative difference between these two preparations was the presence of Sm antigen activity in the Takano preparation which was not present in the Douvas preparation. Takano *et al.* interpreted this finding as indicating that in extracts, nRNP antigen has bound Sm since the antibody used to isolate the nRNP antigen did not react with Sm antigen in a direct precipitin reaction. The conclusion that nRNP is bound to Sm antigen in crude tissue extracts was reached previously by our laboratory based on immunochemical studies with monospecific sera to Sm and nRNP and their ability to absorb their respective antigens from crude tissue extracts (Mattioli & Reichlin, 1973). The differences in results from the two laboratories on the properties of isolated nRNP antigen might well result from the different solvents used to elute the antigen. The pH of the 0·1 N HCl solution used to elute the nRNP in Douvas' studies is known to have destructive

effects on the antigenicity of nRNP. The differences in the nRNP antigenicity, the presence or absence of Sm antigenic activity, and possible differences in subunit structure and composition between the Douvas and Takano preparations may be due in part to the strong acid used to elute the nRNP antigen in the Douvas study. However, other factors may also contribute to these differences and more work is indicated in this area to yield a consistent picture of the antigenic and molecular properties of the nRNP antigen.

Antibodies to the nRNP antigen are more common than antibodies to the Sm antigen amongst SLE patients and in our experience the frequency of these two antibodies as determined by gel diffusion is 30 and 20% respectively. A recent study utilizing the more sensitive counterimmunoelectrophoresis (CIE) technique has put the frequency of antibodies to nRNP and Sm in SLE patients at about 40 and 25% respectively (Tan & Kurata, 1976). As pointed out by our laboratory there is a strong linkage between responses to the two nuclear antigens nRNP and Sm (Mattioli & Reichlin, 1973; Reichlin & Mattioli, 1974). While there are clearly independent antigenic determinants characteristic of these two antigens, there are concomitant immune responses to these two antigens much more frequently than would be expected by chance. Thus sera with two precipitin lines characteristic of nRNP and Sm are more frequent amongst SLE patients than those with only a line against the Sm antigen. Furthermore, on three occasions we have observed patients who for a period had only antibodies to nRNP in their sera but subsequently developed anti-Sm as well, in each case with a concomitant increase in the complexity of the clinical picture.

Recent studies by Lerner and co-workers have added a new dimension and perspective to investigations on the molecular nature of the Sm and nRNP antigens (Lerner & Steitz, 1979; Lerner *et al.*, 1980). Their approach has utilized these antibodies to bind intrinsically labelled RNA protein molecules from Ehrlich ascites tumour cells and then to characterize the particular RNA protein species bound by its molecular weight in urea gels as well as finger printing. Sera of both specificities bind small nuclear RNAs which are heterogeneous and which are known not to be messenger, ribosomal, or transfer RNA. Sera with specificity for nRNP bind a pair of related RNA molecules designated U_{1a} and U_{1b} while sera with specificity for Sm bind U_{1a} and U_{1b} as well as a number of other RNA species, some larger and some smaller than U_{1a} and U_{1b}. The same protein bands are associated with the RNA whether they are found by antibodies to nRNP or Sm. The observation that antibodies to Sm bind everything that anti-nRNP binds as well as additional material is consistent with a heterogeneous substance containing multiple antigenic components as suggested by our previous immunochemical studies (Mattioli & Reichlin, 1973) as well as the more recent immunoaffinity isolation studies (Takano *et al.*, 1980).

The most intriguing aspect of this work lies in the hypothesis about the functional nature of these RNA protein molecules that are bound by the antibodies to the nRNP and Sm antigens. Based on the known base sequences of the RNAs which are complementary to the known consensus base sequences near the junctions of introns and exons it is postulated that these RNA moieties may serve as recognition units for RNA splicing. Indeed, it is conceivable that these RNA protein particles may contain both the recognition units for the splice junctions and/or may contain the splicing enzyme. These studies therefore not only confirm the RNA protein nature of the nRNP antigen, but they also suggest that Sm is of RNP protein nature. Because the antigenicity of Sm is not destroyed by RNase the RNA protein nature of Sm had not been suspected. Moreover, these studies for the first time suggest a biochemical function for these antigens which should heighten further investigation of their role in normal nuclear metabolism as well as suggest new pathways for understanding the pathophysiology of SLE.

The clinical significance of antibodies to nRNP and Sm has been discussed in numerous places and so only a brief summary of that information will be presented. There is agreement amongst workers in this area that antibodies to the Sm antigen are highly specific for SLE patients and serve as marker antibodies for lupus patients (Notman, Kurata & Tan, 1975). No characteristic clinical features are apparent for this group of patients although there are reports that the nephritis of such patients is mild and follows a benign course (Powers *et al.*, 1977; Winn *et al.*, 1979b). It has also been reported that antibodies to Sm are associated with central nervous system disease when it occurs as an isolated clinical manifestation of the disease (Winfield, Brunner & Koffler, 1978).

The presence of antibodies to nRNP as the sole precipitin system demonstrated by the

Ouchterlony technique identifies a subset of lupus patients with a low incidence of antibodies to DNA and a low incidence of clinically apparent renal disease (Sharp *et al.*, 1971; Reichlin & Mattioli, 1972). Patients with ānRNP in their sera develop nephritis only when antibodies of other specificities are present, notably anti-DNA (Parker, 1973; Maddison, Mogavero & Reichlin, 1978). We have also noted that nephritis occurs only when anti-RNP is associated with anti-DNA, anti-Sm, anti-Ro or a combination of antibodies with these latter specificities.

Much attention has centred on a group of patients with overlapping features of SLE, scleroderma and polymyositis who have been designated 'mixed connective tissue disease' (MCTD) by Sharp and his associates (Sharp *et al.*, 1971, 1972). These patients all possess antibodies to nRNP in their sera and, like other patients with anti-nRNP who lack overlapping features, have a low incidence of antibodies to DNA and a low incidence of nephritis.

These patients present with very heterogeneous clinical features, a point noted in the original description and re-emphasized in the recently published follow-up of the original 25 patients (Nimelstein *et al.*, 1980). The only uniform characteristic of these patients is by definition the presence of antibodies to nRNP in their sera.

The author views these patients as that fraction of patients (perhaps 1/5) producing anti-nRNP who have overlapping features of either scleroderma or myositis, the majority behaving as rather typical lupus patients except for a high incidence of Raynaud's phenomenon and a low incidence of nephritis. The ultimate definition of the MCTD syndrome and its nosological relationship to its component connective tissue diseases awaits further clinical, immunogenetic, epidemiological and biochemical investigations.

Alarcón-Segovia and colleagues have recently reported the penetration of antibodies to nRNP into T lymphocytes, particularly Tγ cells, a subpopulation attributed with suppressor activity (Alarcón-Segovia, Ruíz-Arguelles & Fishbein, 1978). The hypothesis has been made that such penetration results in selective depletion of Tγ cells accounting in part for T-suppressor deficiency in these patients. If this work is confirmed it will certainly point to a new, possibly immunopathogenetic, pathway for antibodies reactive with intracellular components.

Antibodies to Ro and La

Antibodies to the soluble antigens Ro and La were originally described in patients with SLE and Sjögren's syndrome (Clark, Reichlin & Tomasi, 1968; Mattioli & Reichlin, 1974). They were thought to be cytoplasmic antigens on the basis of their activity in cytoplasmic extracts of cells prepared with strong sucrose solutions. Purified antibodies to the Ro and La antigens do not stain cryostat sections of mouse liver. Purified antibodies to the Ro antigen do stain the cytoplasm but not the nucleus of KB cells and thymocytes while purified antibodies to the La antigen stain both the cytoplasm and the nucleus of KB cells and thymocytes (Maddison & Reichlin, unpublished experiments). Since the La antigen was destroyed by both RNase and trypsin it was designated an RNA protein antigen; Ro antigen prepared from human spleen extract was resistant to both these enzymes under the same conditions. Antibodies to La are invariably accompanied by anti-Ro (which can and do occur without anti-La). It appears from studies currently underway that patients with both anti-Ro and anti-La have a lower incidence of nephritis and milder disease than those with anti-Ro alone (Wasicek & Reichlin, unpublished data), suggesting important clinical differences between these groups of patients.

Anti-Ro SLE patients who satisfy the preliminary criteria of the ARA are not readily distinguishable from other lupus patients except that a high proportion (75–80%) have a photosensitive dermatitis and positive tests for rheumatoid factors (Maddison *et al.*, 1979). The incidence of other manifestations is similar to other large series of SLE patients or our own SLE patients who lack anti-Ro. In particular, there is at least the expected frequency of nephritis among these patients and in two patients, direct evidence for the participation of the Ro–anti-Ro system via an immune complex mechanism was adduced by the demonstration of enrichment of anti-Ro activity in the glomerular eluates from the nephritic kidneys (Maddison & Reichlin, 1979).

A subset of SLE patients has been described whose sera fail to stain the nuclei of mouse liver cryostat sections in the indirect immunofluorescent test but yet exhibit clinical features consistent

with SLE (Provost *et al.*, 1977; Fessel, 1977; Gladman, Chalmers & Urowitz, 1978). These patients have been designated 'ANA-negative SLE' cases and the serological and clinical features of 66 such patients have been described (Maddison, Provost & Reichlin, 1981). Forty-one of these patients had anti-Ro precipitins in their sera and of these, 21 also had anti-La precipitins, 20 had anti-ssDNA (measured by radioimmunoassay) and only one had a low titre of anti-DNA. Of the remaining 25, 18 had anti-ssDNA and only one had a low titre of anti-nDNA. Thus only five patients had no serological features which linked them to other SLE patients. The failure of anti-ssDNA when it occurs alone to stain the interphase nuclei of epithelial tissue is well known (Tan & Vaughan, 1973).

It was shown that 66% of these sera which were ANA-negative on mouse liver were ANA-positive on KB cells, a cultured human epithelial cell line. This suggests that the liver cells are antigenically deficient for an antigen(s) characteristic of these patients and present in the KB cell line. The clinical picture of these patients was notable for a high incidence of photosensitive dermatitis and rheumatoid factor and a low incidence of nephritis and neuropsychiatric disease. These patients represent 5–10% of the SLE population.

Recently, a subset of lupus patients with a characteristic non-scarring dermatitis designated 'subacute lupus erythematosus' (SCLE) has been described (Sontheimer, Thomas & Gilliam, 1979). The skin disease is intermediate in severity between the acute erythematous eruption of SLE and the chronic indolent scarring lesions of discoid LE. The patients exhibit a wide distribution of lesions with the face, arms and trunk being widely affected. Eighty per cent of these patients are ANA-positive on KB cells and they have a mild systemic illness very similar in character to that which is described in the previous paragraph about our ANA-negative SLE patients. The common factor amongst the ANA-negative SLE patients and the SCLE patients that may relate to their mild disease and the low frequency of nephritis is the low frequency of antibodies to nDNA.

The author's group has also reported that 73% of these SCLE patients are DR3-positive, a great increase above their control frequency of 24% for this particular HLA haplotype (Gilliam & Stastny, 1979). These patients' sera have recently been examined for the presence of antibodies to soluble cellular antigens and it has been found that 67% have antibodies to Ro and/or La antigens (Sontheimer *et al.*, 1980). As mentioned previously, 26% of SLE patients and only 3% of discoid LE patients have anti-Ro in their sera. In a recent report by Bell & Maddison, an even tighter linkage between antibodies to Ro and the HLA haplotype DR3 has been demonstrated (Bell & Maddison, 1980). In their SLE population, all the anti-Ro patients (10/10) were DR3-positive while of the remaining SLE patients without anti-Ro, 28% were positive for the DR3 haplotype. The frequency of DR3 in their control population was 26%. These data suggest that at least part of the clinical heterogeneity of SLE may be due to genetic factors and that one set of genetic determinants may be associated with the production of anti-Ro.

In 1975 Alspaugh & Tan described two precipitating antibody systems in the sera of patients with Sjögren's syndrome which reacted with soluble antigens extracted from Wil_2 cells, a lymphoblastoid B cell line. These were termed 'SSA' and 'SSB' and occurred in 70 and 48% of patients with sicca syndrome alone but in only 9 and 3% respectively of patients with sicca syndrome with rheumatoid arthritis. In their initial studies, none of 27 SLE patients had antibodies to these antigens. Immunofluorescence studies on Wil_2 cells with sera containing anti-SSA and anti-SSB led them to conclude that SSA was a nuclear antigen and that SSB occurred in both the nucleus and the cytoplasm (Alspaugh & Tan, 1975; Alspaugh, Talal & Tan, 1976). Subsequently, Akizuki and co-workers described a soluble nuclear antigen isolated from liver termed Ha, antibodies to which identified a subset of SLE patients with coexistent Sjögren's syndrome as well as the majority of patients with sicca syndrome if a sensitive radioimmunoassay was used to measure antibody in this latter group (Akizuki, Powers & Holman, 1977b; Kassan *et al.*, 1977; Akizuki *et al.*, 1977a). Serum exchanges between groups have revealed that antigenic identity exists between Ro and SSA and that the three antigens La, SSB, and Ha show antigenic identity in double-diffusion experiments in agar gel (Alspaugh & Maddison, 1979). Confusion has resulted from the fact that Ro and La were described as cytoplasmic antigens while SSA, SSB and Ha have all been described as nuclear. In addition, La derived from thymus extracts is RNase-sensitive while the antigenically identical SSB and Ha from Wil_2 cells and liver nuclei respectively are not RNase-sensitive. Since different tissues, extraction methods and conditions of incubation with enzymes were used in these studies, differ-

ences in molecular properties may reflect true molecular differences or differences due to the experimental conditions employed. The immunological identity of these antigens is, however, assured and since methods for antigen purification are rapidly becoming available, the molecular differences (if any) will likely be identified. Akizuki *et al.* (1977a) have already described an immunoaffinity procedure for purification of Ha (La, SSB) which results in 550-fold purification and a material suitable for radioiodination and radioimmunoassay.

Subsequent clinical studies of the SSA (Ro) system in a larger group of SLE patients has shown that 26% of such patients have anti-SSA (anti-Ro) and that in some patients antibody levels may fluctuate with disease activity (Scopelitis, Biundo & Alspaugh, 1980).

Molecular biological studies with sera containing anti-Ro and anti-La specificities by Lerner *et al.* (1981) have shown that such sera bind a set of small RNAs designated Y_2, Y_3 and Y_4 and which are different from the RNAs bound by the antibodies which bind the Sm and nRNP antigens. Presumably, these RNAs are part of an RNA protein complex since the antigenic activity of La (SSB, Ha) is trypsin-sensitive as is SSA (Ro) derived from Wil_2 extracts. The RNA bands are different in size and sequence from those which bind Sm and nRNP. There is as yet no known function of these small RNAs which bind Ro and La. Again it is of interest that antibodies to Ro antigens may bind RNA or RNA protein complexes since this was unexpected due to the lack of RNase on Ro (SSA) antigenicity.

These antibodies are therefore characteristic of Sjögren's syndrome and a subset of SLE patients most of whom do not have sicca symptoms. Although the first reports heralded the specificity of anti-SSA for isolated sicca or 'primary' Sjögren's with rheumatoid arthritis this has not been found in a recent study in which such antibodies occurred equally frequently in the two groups (Alexander *et al.*, 1980).

Antibodies to PCNA and Ma

Two recently-described antibodies which occur in small groups of SLE patients are of interest for various reasons. PCNA is an abbreviation for proliferating cell nuclear antigen described by Miyachi *et al.* (1978). This antigen is not only seen in interstitial cells in epithelial tissues like liver but is seen in the majority of cells of actively proliferating tissues such as spleen. The important point here is that antibodies may exist in SLE sera which are directed to antigens not well represented in tissues used as standard immunofluorescent substrates such as liver and kidney. A similar situation has been shown in recent studies with scleroderma sera in which the tissue culture line Hep_2 proved to be a particularly favourable substrate for the detection of antibodies in scleroderma sera (Tan *et al.*, 1980).

The Ma antigen is an acidic nuclear protein described by Winn *et al.* (1979a), antibodies to which occur in a subset (approx. 10%) of SLE patients with particularly severe disease. Of interest, if confirmed, is the association of this particular antibody with aggressive active disease and nephritis. Evidence was also presented demonstrating the alternating presence of antigen and antibody in the patients' sera suggesting that immune complexes involving this system may be forming in the circulation of these patients.

CONCLUSION

Much progress has been made in the past several years in the immunochemistry and immunopathology of the DNA–anti-DNA system and promising initial investigations have been made in the isolation and chemical characterization of the non-DNA-containing antigens. In addition, continued molecular biological, immunogenetic and clinical studies can only enhance our understanding of these antigen–antibody systems and illuminate the relationship of the synthesis of these antibodies to the basic pathogenesis of SLE.

Research by the author of this article is supported by research grants NIH AM 10428 and NIH AM 21750, and by research funds of the Veterans Administration.

REFERENCES

AARDEN, L.A., DEGROOT, E.R. & FELTKAMP, T.E.W. (1975) Immunology of DNA. III. *Crithidia luciliae*, a simple substrate for the determination of anti-dsDNA with the immunofluorescent technique. *Ann. NY Acad. Sci.* **254,** 505.

AKIZUKI, M., BOEHM-TRUITT, M.J., KASSAN, S.S., STEINBERG, A.D. & CHUSED, T.M. (1977a) Purification of an acidic nuclear protein antigen and demonstration of its antibodies in subsets of patients with sicca syndrome. *J. Immunol.* **119,** 932.

AKIZUKI, M., POWERS, R. & HOLMAN, H.R. (1977b) A soluble acidic protein of the cell nucleus which reacts with serum from patients with systemic lupus erythematosus and Sjögren's syndrome. *J. clin. Invest.* **59,** 264.

ALARCÓN-SEGOVIA, D., RUÍZ-ARGUELLES, A. & FISHBEIN, E. (1978) Antibody to nuclear ribonucleoprotein penetrates live human mononuclear cells through Fc receptors. *Nature*, **271,** 67.

ALEXANDER, E., HIRSCH, T., ARNETT, F.C., PROVOST, T.T. & STEVENS, M.B. (1980) The clinical spectrum of anti-Ro antibody. *Arthritis Rheum.* **23,** 648 (abstract).

ALSPAUGH, M.A. & MADDISON, P.J. (1979) Resolution of the identity of certain antigen–antibody systems in systemic lupus erythematosus and Sjögren's syndrome: an interlaboratory collaboration. *Arthritis Rheum.* **22,** 796.

ALSPAUGH, M.A., TALAL, N. & TAN, E.M. (1976) Differentiation and characterization of autoantibodies and their antigens in Sjögren's syndrome. *Arthritis Rheum.* **19,** 216.

ALSPAUGH, M.A. & TAN, E.M. (1975) Antibodies to cellular antigens in Sjögren's syndrome. *J. clin. Invest.* **55,** 1067.

ANDERSON, J.R., GRAY, K.G., BECK, J.S. & KINNEAR, W.F. (1961) Precipitating autoantibodies in Sjögren's disease. *Lancet*, **ii,** 456.

ANDRES, G.A., ACCINNI, G.A., BEISER, S.M., CHRISTIAN, C.L., CINOTTI, G.A., ERLANGER, B.F., HSU, K.C. & SEEGAL, B.C. (1971) Localization of fluorescein-labelled antinucleoside antibodies in glomeruli of patients with systemic lupus erythematosus nephritis. *J. clin. Invest.* **49,** 2106.

BARNETT, E.V. (1968) Detection of nuclear antigens (DNA) in normal and pathologic human fluids by quantitative complement fixation. *Arthritis Rheum.* **11,** 407.

BEAULIEU, A., QUISMORIO, F.P., FRIOU, G.J., VAYUVEGULA, B. & MIRICK, B. (1979) IgG antibodies to double stranded DNA in systemic lupus erythematosus sera: independent variation of complement fixing activity and total antibody content. *Arthritis Rheum.* **22,** 565.

BELL, D.A. & MADDISON, P.J. (1980) Serologic subsets in SLE: an examination of autoantibodies in relationship to clinical features of disease and HLA antigens. *Arthritis Rheum.* **23,** 1268.

CLARK, G., REICHLIN, M. & TOMASI, T.B. (1968) Characterization of a soluble cytoplasmic antigen reactive with sera from patients with systemic lupus erythematosus. *J. Immunol.* **102,** 117.

COCHRANE, C.G. & KOFFLER, D. (1973) Immune complex disease in experimental animals and man. *Adv. Immunol.* **16,** 185.

DOUVAS, A.S., STUMPH, W.E., REYES, P. & TAN, E.M. (1979) Isolation and characterization of nuclear ribonucleoprotein complexes using human antinuclear ribonucleoprotein antibodies. *J. biol. Chem.* **254,** 3608.

FESSEL, W.J. (1977) ANA negative systemic lupus erythematosus. *Am. J. Med.* **64,** 80.

GILLIAM, J.N. & STASTNY, P. (1979) Subacute cutaneous lupus erythematosus, a form of lupus erythematosus associated with HLA-B-8 and DRw3. *Arthritis Rheum.* **22,** 612 (abstract).

GLADMAN, D.D., CHALMERS, A. & UROWITZ, M.B. (1978) Systemic lupus erythematosus with negative LE cells and antinuclear factor. *J. Rheumatol.* **5,** 142.

HARGRAVES, M.M., RICHMOND, H. & NORTON, R. (1948) Presentation of two bone marrow elements: the 'tart' cell and the 'LE' cell. *Mayo Clin. Proc.* **23,** 25.

KASSAN, S.S., AKIZUKI, M., STEINBERG, A.D., REDDICK, R.L. & CHUSED, T.M. (1977) Antibody to a soluble nuclear antigen in Sjögren's syndrome. *Am. J. Med.* **63,** 328.

KOFFLER, D., AGNELLO, V. & KUNKEL, H.G. (1974) Polynucleotide immune complexes in serum and glomeruli of patients with systemic lupus erythematosus. *Am. J. Pathol.* **74,** 109.

KOFFLER, D., AGNELLO, V., THOBURN, R. & KUNKEL, H.G. (1971a) Systemic lupus erythematosus: prototype of immune complex nephritis in man. *J. exp. Med.* **134,** 109.

KOFFLER, D., AGNELLO, V., WINCHESTER, R.V. & KUNKEL, H.G. (1973) The occurrence of single stranded DNA in the serum of patients with systemic lupus erythematosus and other diseases. *J. clin. Invest.* **52,** 198.

KOFFLER, D., CARR, R.I., AGNELLO, V., THOBURN, R. & KUNKEL, H.C. (1971b) Antibodies to polynucleotides in human sera: antigenic specificity and relation to disease. *J. exp. Med.* **134,** 294.

KOFFLER, D., SCHUR, P.H. & KUNKEL, H.G. (1967) Immunological studies concerning the nephritis of systemic lupus erythematosus. *J. exp. Med.* **126,** 607.

LERNER, M.R., BOYLE, J.A., HARDIN, J. & STEITZ, J.A. (1981) Two novel classes of small ribonucleoproteins detected by antibodies associated with systemic lupus erythematosus. *Science.* (In press.)

LERNER, M., BOYLE, J.A., MOUNT, S.M., WOLIN, S.L. & STEITZ, J.A. (1980) Are snRNPs involved in splicing? *Nature*, **283,** 220.

LERNER, M.R. & STEITZ, J.A. (1979) Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. (USA)*, **76,** 5495.

LIEU, T.A. & TAN, E.M. (1979) Purification of a nuclear non histone protein (Sm antigen) reacting with the sera of patients with systemic lupus erythematosus. *Arthritis Rheum.* **22,** 635 (abstract).

LOCKER, J.D., MEDOF, M.E., BURNETT, R.M. & SUKHUPUNYARASKA, S. (1977) Characterization 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.* **118,** 694.

MADDISON, P.J., MOGAVERO, H., PROVOST, T.T. & REICHLIN, M. (1979) The clinical significance of autoantibodies to a soluble cytoplasmic antigen in systemic lupus erythematosus and other connective tissue diseases. *J. Rheumatol.* **6,** 189.

MADDISON, P.J., MOGAVERO, H. & REICHLIN, M. (1978) Patterns of clinical disease associated with antibodies to nuclear ribonucleoprotein. *J. Rheumatol.* **5,** 407.

MADDISON, P.J., PROVOST, T.T. & REICHLIN, M. (1981) ANA-negative systemic lupus erythematosus: serological analysis. *Medicine (Baltimore).* (In press.)

MADDISON, P.J. & REICHLIN, M. (1979) Deposition of antibodies to a soluble cytoplasmic antigen in the kidneys of patients with systemic lupus erythematosus. *Arthritis Rheum.* **22,** 858.

MATTIOLI, M. & REICHLIN, M. (1971) Characterization of a soluble nuclear ribonucleoprotein antigen reactive with SLE sera. *J. Immunol.* **197,** 1281.

MATTIOLI, M. & REICHLIN, M. (1973) Physical association of two nuclear antigens and mutual occurrence of their antibodies: the relationship of the Sm and RNA protein (Mo) systems in SLE sera. *J. Immunol.* **110,** 1318.

MATTIOLI, M. & REICHLIN, M. (1974) Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus. *Arthritis Rheum.* **17,** 421.

MINITER, M.F., STOLLAR, B.D. & AGNELLO, V. (1979) Reassessment of the clinical significance of native DNA antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **22,** 959.

MIYACHI, I., FRITZLER, M.J. & TAN, E.M. (1978) Autoantibody to a nuclear antigen in proliferating cells. *J. Immunol.* **121,** 2228.

NIMELSTEIN, S.H., BRODY, S., MCSHANE, D. & HOLMAN, H.R. (1980) Mixed connective tissue disease: a subsequent evaluation of the original 25 patients. *Medicine (Baltimore),* **59,** 239.

NOTMAN, D.D., KURATA, N. & TAN, E.M. (1975) Profiles of antinuclear antibodies in systemic rheumatic disease. *Ann. intern. Med.* **83,** 464.

PAPALIAN, M., LAFER, E., WONG, R. & STOLLAR, B.D. (1980) Reaction of systemic lupus erythematosus antinative DNA antibodies with native DNA fragments from 20–1200 base pairs. *J. clin. Invest.* **65,** 469.

PARKER, M.D. (1973) Ribonucleoprotein antibodies: frequency and clinical significance in systemic lupus erythematosus, scleroderma, and mixed connective tissue disease. *J. lab. clin. Med.* **82,** 769.

POWERS, R., AKIZUKI, M., BOEHM-TRUITT, M.J., DALY, V. & HOLMAN, H.R. (1977) Substantial purification of the Sm antigen and association of high titer antibody to Sm with a clinical subset of systemic lupus erythematosus. *Arthritis Rheum.* **20,** 131 (abstract).

PROVOST, T.T., AHMED, A.R., MADDISON, P.J. & REICHLIN, M. (1977) Antibodies to cytoplasmic antigens in cutaneous lupus: serological marker for systemic disease. *Arthritis Rheum.* **20,** 1457.

REICHLIN, M. & MATTIOLI, M. (1972) Correlation of a precipitin reaction to an RNA protein antigen and a low prevalence of nephritis in patients with systemic lupus erythematosus. *N. Engl. J. Med.* **286,** 908.

REICHLIN, M. & MATTIOLI, M. (1974) Antigens and antibodies characteristic of systemic lupus erythematosus. *Bull. rheum. Dis.* **24,** 756.

SAMAHA, R.J. & IRVIN, W.S. (1975) Deoxyribonucleic acid strandedness: partial characterization of the antigenic region binding antibodies in lupus erythematosus serum. *J. clin. Invest.* **56,** 446.

SCHUR, P.M. & SANDSON, J. (1968) Immunologic factors and clinical activity in systemic lupus erythematosus. *N. Engl. J. Med.* **278,** 533.

SCOPELITIS, E., BIUNDO, J.J. & ALSPAUGH, M.A. (1980) Anti-SSA antibody and other antinuclear antibodies in systemic lupus erythematosus. *Arthritis Rheum.* **23,** 287.

SHARP, G.C., IRVIN, W.S., LAROQUE, VELEZ, C., DALY, V., KAISER, A.D. & HOLMAN, H.R. (1971) Association of autoantibodies to different nuclear antigens with clinical patterns of rheumatic disease and responsiveness to therapy. *J. clin. Invest.* **50,** 350.

SHARP, G.C., IRVIN, W.S., TAN, E.M., GOULD, R.G. & HOLMAN, H.R. (1972) Mixed connective tissue disease – an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am. J. Med.* **52,** 148.

SONTHEIMER, R.D., STASTNY, P., MADDISON, P., REICHLIN, M. & GILLIAM, J.N. (1980) AntiRo and antiLa antibodies and further HLA associations in subacute cutaneous lupus erythematosus. *Arthritis Rheum.* **23,** 80 (abstract).

SONTHEIMER, R.D., THOMAS, J.R. & GILLIAM, J.N. (1979) Subacute cutaneous lupus erythematosus. A cutaneous marker for a distinct lupus erythematosus subset. *Arch. Dermatol.* **115,** 1409.

STOLLAR, B.D. (1973) Nucleic acid antigens. In *The Antigens* (ed. by M. Sela), Vol. 1. Academic Press, New York and London.

STOLLAR, B.D. & PAPALIAN, M. (1980) Secondary structure in denatured DNA is responsible for its reaction with antinative DNA antibodies of systemic lupus erythematosus sera. *J. clin. Invest.* **66,** 210.

TAKANO, M., AGRIS, P.F. & SHARP, G.C. (1980) Purification and biochemical characterization of nuclear ribonucleoprotein antigen using purified antibody from serum of a patient with mixed connective tissue disease. *J. clin. Invest.* **65,** 1449.

TAN, E.M. & KUNKEL, M.G. (1966) Characteristics of a soluble nuclear antigen precipitating with the sera of patients with systemic lupus erythematosus. *J. Immunol.* **96,** 464.

TAN, E.M. & KURATA, N. (1976) Identification of antibodies to nuclear acidic antigens by counterimmunoelectrophoresis. *Arthritis Rheum.* **19,** 574.

TAN, E.M., RODNAN, G.P., GARCIA, I., MOROI, Y., FRITZLER, M.J. & PEEBLES, C. (1980) Diversity of antinuclear antibodies in progressive systemic sclerosis. *Arthritis Rheum.* **23,** 617.

TAN, E.M., SCHUR, P.H. & CARR, R.I. (1966) Deoxyribonucleic acid (DNA) and antibodies to DNA in the serum of patients with systemic lupus erythematosus. *J. clin. Invest.* **45,** 1732.

TAN, E.M. & VAUGHAN, J.H. (1973) Antinuclear antibodies: significance of biochemical specificities. In *Immunopathology of the Skin. Labeled Antibody Studies* (ed. by E. H. Beutner, T. P. Charezelski, S.

F. Bean and R. E. Jordan), p. 367. Dowden, Hutchison & Ross, Stroudsburg, Pennsylvania.

WINFIELD, J.B., BRUNNER, C.M. & KOFFLER, D. (1978) Serologic studies in patients with systemic lupus erythematosus and central nervous system dysfunction. *Arthritis Rheum.* **21,** 289.

WINFIELD, J.B., KOFFLER, D. & KUNKEL, H.G. (1975) Specific concentration of polynucleotide immune complexes in the cryoprecipitates of patients with systemic lupus erythematosus. *J. clin. Invest.* **56,** 563.

WINN, D.M., WOLFE, J.F., HARMON, D. & SHARP, G.C. (1979a) Characterization of a distinct nuclear acidic protein antigen (MA) and clinical findings in systemic lupus erythematosus patients with MA antibodies. *J. clin. Invest.* **64,** 820.

WINN, D.M., WOLFE, J.F., LINDBERG, D.A., FRISTOE, F.A., KINGSLAND, L. & SHARP, G.C. (1979b) Identification of a clinical subset of systemic lupus erythematosus by antibodies to the Sm antigen. *Arthritis Rheum.* **22,** 1334.