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AUTOANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

Ingrid Helena Olhoffer

Yale University

1995

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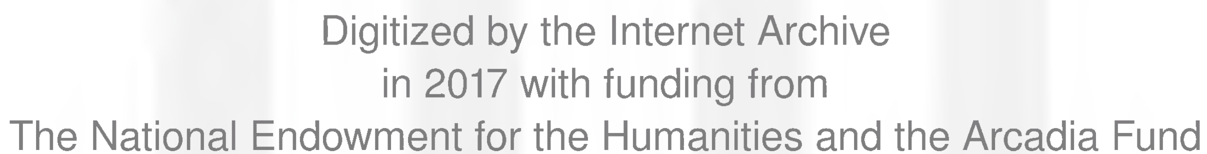

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AUTOANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine

by

Ingrid Helena Olhoffer

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ABSTRACT

AUTOANTIBODY PROFILES IN SYSTEMIC LUPUS ERYTHEMATOSUS SUPPORT A GLOBAL DEFECT IN IMMUNE TOLERANCE. Ingrid H. Olhoffer and Joseph Craft. Section of Rheumatology, Department of Internal Medicine, Yale University, School of Medicine, New Haven, CT.

Systemic lupus erythematosus (SLE) is manifested by a diversity of both clinical manifestations and autoantibody specificities. Previous studies have suggested that autoantibody specificities in SLE occur in pairs (dsDNA and histone, Ro and La, Sm and U1 ribonucleoprotein). This led to the hypothesis that the autoimmune response in lupus targets particles - the nucleosome, the Ro/La ribonucleoprotein particle, and/or the spliceosome. Given this theory, we studied the frequency of autoantibody sets and the average number of autoantibody sets per SLE patient in regard to the etiology of lupus. Sera of sixty-eight patients fulfilling the American Rheumatology Association 1982 Revised Criteria for SLE were studied for autoantibody sets by ^{35}S immunoprecipitation and ELISA analysis, and autoantibody profiles previously reported in the literature were examined for the prevalence of autoantibody sets and the average number of autoantibody sets per patient. Clinical/autoantibody associations, in this previously unreported population, were determined using Chi-square analysis with Yates' correction. The prevalences of autoantibody sets of the following specificities were as follows: dsDNA and/or histone (59%), Sm and/or U1 RNP (40%), and Ro and/or La (41%). The current study and twelve identified studies in the literature showed an average of two or greater autoantibody sets per patient supporting lupus etiologic theories consistent with a global defect in immune tolerance. The following

associations, which are consistent with the literature, were found - anti-dsDNA antibodies correlated with renal pathology; anti-dsDNA and anti-histone antibodies correlated with hypocomplementemia; and anti-La antibodies correlated with Rheumatoid Factor.

ABBREVIATIONS

ANA	Antinuclear antibody
ARA	American Rheumatology Association
BSA	Bovine serum albumin
CIE	Counterimmunoelectrophoresis
DNA	Deoxyribonucleic acid
ds	Double-stranded
EDTA	Ethylenediamine-tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
HLA	Human lymphocyte antigen
IL	Interleukin
IPP	Immunoprecipitation
kD	Kilodalton
lpr	Lymphoproliferation
MCTD	Mixed connective tissue disease
MHC	Major histocompatibility complex
MRL	Murphy's recombinant large
mRNA	Messenger ribonucleic acid
MW	Molecular weight
nRNP	Nuclear ribonucleoprotein
NZB	New Zealand Black
NZW	New Zealand White
PBCA	Polyclonal B cell activation
PCNA	Proliferating cell nuclear antigen
PTCA	Polyclonal T cell activation
RF	Rheumatoid factor

RNA	Ribonucleic acid
RNP	Ribonucleoprotein
rRNA	Ribosomal ribonucleic acid
SCLE	Subacute cutaneous lupus erythematosus
scRNP	Small cytoplasmic ribonucleoprotein
SD	Standard difference
SDS	Sodium dodecyl sulfate
SLE	Systemic lupus erythematosus
Sm	Smith
snRNP	Small nuclear ribonucleoprotein
ss	Single-stranded
SS	Sjögren's syndrome
T_h	Helper T cell
tRNA	Transfer ribonucleic acid

INTRODUCTION

Systemic lupus erythematosus (SLE) is manifested by a diversity of both clinical manifestations and autoantibody specificities. Clinical manifestations include: dermatologic disease, serositis, musculoskeletal disease, neurological disease, vascular disease, renal pathology, hematologic disease, and constitutional symptoms (Tan et al., 1982). Autoantibodies in SLE can be artificially divided into organ specific versus organ non-specific antibodies (Tan 1993). The organ specific antibodies are directed against antigens present in only a subset of cells, for example, the hematopoietic cellular elements (red cells, white cells, and platelets) (Tan 1993); whereas organ non-specific antibodies (the focus of this project) are directed against subcellular nuclear and cytoplasmic antigens common to virtually every cell type, including double-stranded (ds) DNA, histones, Sm, U1 ribonucleoprotein (RNP), Ro, La, ribosomal proteins, proliferating cell nuclear antigen (PCNA), Ku, and Ki (Boey et al., 1988). These autoantibodies have been used as markers to confirm diagnosis, shown to play a direct role in the pathogenesis of disease, correlated with clinical manifestations, used as tools for the structural analysis of the autoantigens, and examined for clues to the etiology of lupus. Possible etiologies include: modification of self determinants, molecular mimicry, polyclonal B cell activation (PBCA), polyclonal T cell activation (PTCA), disclosure of "privileged sites," disclosure of "cryptic self," activation of ignorant autoreactive cells, a deficit in T and/or B cell tolerance, or an immunoregulatory disturbance (Theofilopoulos 1995). The present study takes a novel approach to examining autoantibody profiles for clues to the etiology of SLE. In order to address this question, the following introduction

will briefly detail the history of the autoantibodies, the molecular biology of the autoantigens, the methods for detection of autoantibodies, the clinical associations of the autoantibodies, and the various theories regarding the genesis of autoantibodies.

History of Autoantibodies in SLE

Serological markers of the connective tissue diseases have an extended history of being studied. In 1948, Hargraves, one of the pioneers in laboratory rheumatology, described the phenomenon of the LE (lupus erythematosus) cell in bone marrow and associated it with SLE (Hargraves et al., 1948). Shortly thereafter, Kunkel and colleagues showed that the LE phenomenon was secondary to circulating antibodies against DNA, cell nuclei and deoxyribonucleoprotein (which direct phagocytosis by polymorphonuclear leukocytes), giving new insight into the immunopathogenesis of SLE and shaping the development of modern concepts of autoimmunity (reviewed in Tan 1989; and Christian and Elkon, 1986).

Since these early studies led the way for other investigators, many other autoantibodies have been identified. In 1959, antibodies to DNA were first reported (reviewed in Tan 1993). In 1961, Kunkel et al. described anti-histone antibodies (reviewed in Christian and Elkon, 1986). Discovery of the small nuclear ribonucleoprotein (snRNP) particles began in the early 1960s with anti-Sm being first described in SLE patients in 1966 by Tan and Kunkel and nRNP (now called U1 RNP) being described in 1971 (reviewed in Hardin 1989, and Mattioli and Reichlin, 1971). Antibodies to ribosomes were first described in 1963 (reviewed in Bonfa and Elkon, 1986). Anti-Ro

and anti-La antibodies were first reported to occur in patients with SLE in 1968 (reviewed in Reichlin, 1985), and in 1979, an interlaboratory collaboration showed these antigens to be equal to the SS-A and SS-B antigens, respectively (SS-A and SS-B were originally described in Sjögren's syndrome) (Alspaugh and Maddison, 1979). An "autoantibody to a nuclear antigen in proliferating cells" (PCNA) was first reported in 1978 (Miyachi et al., 1978).

Discoveries continued into the 1980s. Anti-Ku antibodies, which were first isolated in patients with polymyositis-scleroderma overlap syndrome, were isolated in SLE patients in 1981 (reviewed in Reeves 1985). Also in 1981, Tono et al. found that SLE patients have antibodies which target the Ki antigen (reviewed in Sakamoto et al., 1989). The SL antigen, first reported by Harmon et al. in 1981, has since been shown to be equivalent to Ki (Bernstein et al., 1986; Sakamoto et al., 1989).

Also of historical importance, many autoantibody specificities were discovered using patient sera and were therefore originally named after the patient in whom they were first described (Christian and Elkon, 1986). For example, anti-Sm and anti-Ku antibodies were first described in the sera of patients Smith and Ku (Craft and Hardin, 1992; Christian and Elkon, 1986).

Characteristics of Autoantigens in SLE

Using patient autoantibodies as probes together with recent advances in molecular biology, investigators have accumulated a considerable amount of knowledge regarding the molecular identity and biological functions of the autoantigens targeted in lupus (Table 1).

DNA Many patients with SLE have sera specific for their genetic material, deoxyribonucleic acid (DNA). DNA is a polymer of deoxyribonucleotides whose function can be described as "the storehouse of information specifying all facets of the cell's existence (Geis 1983)". Much research has described the antigenic determinants recognized by anti-DNA antibodies. Antibodies to dsDNA react with antigenic determinants present on both double and single-stranded (ss) DNA (mainly the deoxyribose phosphate backbone) (reviewed in Tan 1993), whereas, antibodies to ssDNA target the purine and pyrimidine bases exposed in ssDNA (reviewed in Tan 1989 and Tan 1993).

Histones Histones are a highly conserved family of basic proteins within the nucleus which together with DNA make up nucleosomes. Nucleosomes are highly structured units consisting of H2A-H2B dimers and H3-H4 tetramers forming a core structure around which helices of dsDNA are wound (reviewed in Tan 1989). H1 proteins mediate a higher order of packing (reviewed in Craft and Hardin, 1992). In SLE, autoantibodies to histones are targeted at all the classes of histones, H1, H2A, H2B, H3, H4, as well as the H2A/H2B and H3/H4 complexes (reviewed in Tan 1989 and Tan 1993).

snRNP Antigens In 1979, Lerner and Steitz first elucidated the structure and function of these antigens which are classified as small nuclear ribonucleoprotein (snRNP) particles - U1, U2, and U4-U6. Structurally, each spliceosome snRNP particle consists of the corresponding U (uridine rich) small RNA (U1, U2, U4, U5, or U6), distinct from tRNA, mRNA and rRNA, and associated polypeptides. The Sm protein complex consists of

six polypeptides B (28 kD), B' (29 kD), D (16 kD), and E-G (13-11 kD) which are common to the U1, U2, and U4-U6 snRNPs; whereas, the 70K (70 kD), A (33 kD), and C (22 kD) polypeptides are uniquely associated with the U1 snRNP. (reviewed in Tan 1989; and Craft et al., 1988; and Hardin 1986)

Functionally, snRNP particles are involved in splicing pre-mRNA as a part of the spliceosome machinery located in the nucleus. The proteins of these particles have most commonly been shown to be the immune target; however, antibodies to the RNA component have also been identified (Wilusz and Keene, 1986). Specifically, anti-U1 RNP antibodies bind 70K, A, and/or C; anti-Sm antibodies bind B, B', D and/or E recognizing one or more epitopes (they may also bind F and/or G) (reviewed in Tan 1989); and anti-U2 RNP antibodies bind A' and B" (Craft et al., 1988). E, F and G are rarely autoimmune targets (reviewed in Tan 1989, and Hardin 1989).

Other important aspects of the snRNP binding specificities have been elucidated. For example, there is a shared conformational epitope on B'/B and D recognized by a monoclonal antibody called Y12. Furthermore, it has been noted that several epitopes are common to the various peptides and they have therefore been suggested as "pivotal" in the SLE autoimmune response. (reviewed in Hardin 1989)

Ro Anti-Ro/SS-A positive sera bind ribonucleoprotein particles. Each particle consists of probably two Ro proteins (52 kD and 60 kD) and 4-5 small RNAs called Y RNAs (reviewed in Tan 1989; and Craft and Hardin, 1992). The Ro RNAs are transcribed by RNA polymerase III. Initially thought to be cytoplasmic, Ro antigen is now believed to be nuclear in origin (Clark et al., 1969). The function of Ro is unknown.

La La/SS-B is a conserved phosphoprotein of 46 to 48 kD. Gottlieb and Steitz in 1989, suggested that La is a RNA polymerase III termination factor (Gottlieb and Steitz, 1989). Interestingly, anti-La antibodies have been shown to target an antigen present on both the Ro and La particles. (reviewed in Tan 1989)

Ribosomal P Proteins Antibodies to ribosomal ribonucleoprotein (rRNP) are directed against three proteins, P0, P1, and P2 (of 38 kD, 16 kD, and 15 kD, respectively) of the 60S ribosomal subunit (Elkon et al., 1988). As a part of the translation machinery, these proteins are at least indirectly involved in mRNA translation and protein synthesis. Accordingly, on ANA immunofluorescence, staining for these proteins is seen in both the nucleolus and cytoplasm (reviewed in Craft and Hardin, 1992).

Ku Ku, also called p70/p80, is represented by a doublet of proteins of 60-66 kD and 81-86 kD (reviewed in Tan 1989; Sakamoto et al., 1989). At least three epitopes (one common to both proteins) have been identified (Francoeur et al 1986). Ku is the regulatory component of a DNA-dependent protein kinase (Dvir et al., 1992, Gottlieb and Jackson, 1993).

Ki Ki is a nuclear protein of 32 kD whose function is unknown (Sakamoto et al., 1989).

PCNA As a 36 kD cell cycle-regulated proliferation-associated protein, PCNA is used as a probe for identifying proliferating cells and has been reported to be the auxiliary protein of DNA polymerase-delta (Miyachi et al., 1978; reviewed in Craft and Hardin, 1992; Swaak et al., 1990).

Detection of Autoantibodies

Many techniques have been used to study the serology of SLE including: tissue section immunofluorescence, cell substrate immunofluorescence, Farr radioimmunoassay, Crithidia immunoassay, immunodiffusion, counterimmunoelectrophoresis, ELISA, western blotting, and immunoprecipitation. Throughout the years, many seemingly conflicting studies of SLE autoantibodies have used different assays. Accordingly, much of the discordant data in the literature may be explained by differences in assay sensitivities and specificities.

ANA Immunofluorescence Antinuclear antibody immunofluorescence is the most prevalent screening assay for the detection and quantification of ANAs. Tissue section immunofluorescence and cell substrate immunofluorescence are sensitive, nonspecific screens for autoantibodies which are usually followed by more specific antibody tests (Christian and Elkon, 1986).

Anti-dsDNA Detection The common techniques for anti-dsDNA detection include the Farr radioimmunoassay, the Crithidia immunofluorescence assay, and the ELISA (Christian and Elkon, 1986). The Farr assay, described in 1968, is based on separating free DNA from DNA-antibody complexes using saturated ammonium sulfate solutions (Wold et al., 1968). The Crithidia assay uses a stage double-stranded solid phase form of DNA for semiautomated immunofluorescent intensity quantification (Christian and Elkon, 1986). ELISAs (enzyme-linked immunosorbent assays) involve

using purified antigen as bound substrate which is then probed with unknown sera, with bound antibodies being detected with labeled anti-IgG antibodies. Studies comparing various assays have found varying sensitivities and specificities (reviewed in Craft and Hardin, 1992). In a study by Swaak et al., sensitivities and specificities varied depending on the clinical manifestations of the SLE patient population. In particular, Swaak et al. showed qualitative and quantitative differences between patients with CNS lupus manifestations and nephritis (Swaak et al., 1990).

Immunodiffusion Sm, U1 RNP, Ro and La antisera were originally detected by immunodiffusion analysis, according to Ouchterlony. In this assay, the presence of autoantibodies results in the formation of a visible precipitin line between antigen of various mammalian tissue extracts and specific antibody (reviewed in Craft and Hardin, 1992; and Tan 1991). This technique, which has also been used to detect anti-PCNA (Boey et al., 1988) and anti-Ku antibodies, is dependent on antigen solubility (detecting only precipitating antibodies), antigen abundance, and protein half-life (reviewed in Christian and Elkon, 1986; and Tan 1991). Specifically, Sm, U1 RNP, and La tend to be highly abundant and soluble in most mammalian tissue, whereas Ro is present in lower concentrations in species such as rats or mice (reviewed in Christian and Elkon, 1986). Because the Ro antigen varies in concentration in cells from one animal species to the next, many traditionally “ANA-negative” lupus patients have anti-Ro (reviewed in Christian and Elkon, 1986). A newer technique related to immunodiffusion is counterimmunoelectrophoresis (CIE) (reviewed in Christian and Elkon, 1986).

ELISA Described above, ELISAs are currently used both in research and clinically to screen for specific autoantigens. Numerous studies have been done comparing various detection assays with ELISAs. Maddison et al. showed that ELISA has a greater sensitivity but lower specificity in comparison with immunodiffusion for nRNP, Sm, Ro and La (Maddison et al., 1985). Anti-histone antibodies are usually detected with ELISA because histones are insoluble at physiologic ionic strength and pH. (reviewed in Christian and Elkon, 1986)

Immunoprecipitation (IPP) (See methods section for description of technique.) Currently one of the most sensitive and specific methods for characterizing the targets of autoantibodies is immunoprecipitation (reviewed in Craft and Hardin, 1992; and Christian and Elkon, 1986). Kessler was one of the first to use the technique of immunoprecipitation (reviewed in Christian and Elkon, 1986), and in 1979, Lerner and Steitz used this technique to immunoprecipitate radiolabeled RNA protein complexes (Lerner and Steitz, 1981). Immunoprecipitation to detect anti-snRNP, anti-Ro, and anti-La antibodies is well described (Craft and Hardin, 1992). Immunoprecipitation has also been used to identify antibodies to rRNP, Ki and Ku (Boey et al., 1988). One of the important elements of this technique is that non-antigenic proteins and RNA associated with the targeted antigen are co-precipitated. Immunoprecipitation also has utility in detecting previously unrecognized antigens.

Western Blotting Western blotting involves probing protein which has been transferred to nitrocellulose paper after SDS gel separation. It is a very

specific way of identifying protein antigens (reviewed in Christian and Elkon, 1986). Bonfa and Elkon showed that Western blotting was the most sensitive and specific method for the detection of anti-ribosomal P protein antibodies in comparison to CIE and cytoplasmic indirect immunofluorescence (Bonfa and Elkon, 1986).

Prevalence of Autoantibodies in SLE

Frequencies of Individual Autoantibody Specificities The frequency of particular autoantibodies found in a given SLE population is assay dependent; accordingly, many varying percentages have been reported (Table 8). Nevertheless, some generally accepted frequencies are quoted in the literature (Table 1). Studies have shown that approximately 40% of SLE patients have anti-dsDNA antibodies, and a considerably higher percentage, approximately 70%, have anti-ssDNA antibodies (reviewed in Tan 1989). Approximately 70% of SLE patients have been shown to have anti-histone antibodies (reviewed in Tan 1989). Anti-Sm and anti-U1 RNP antibodies are found in approximately 15-30% and 32% of SLE patients, respectively (Boey et al., 1988; reviewed in Tan 1989; Swaak et al., 1990). Anti-Ro antibodies are present in approximately 35% of SLE patients and anti-La antibodies are found in approximately 15% of patients (reviewed in Tan 1989). At least 10-15% of patients with SLE have antibodies reactive with the ribosomal P proteins (Christian and Elkon, 1986; Elkon et al., 1988). Anti-Ku antibodies occur in approximately 5-10% of SLE patient sera (Boey et al., 1988; reviewed in Tan 1989; Sakamoto et al., 1989), and 6-21% of SLE patients have antibodies directed against the 32 kD Ki/SL antigen (Boey et al., 1988; Bernstein et al., 1984; Reichlin 1985; Swaak et al., 1990). Anti-

PCNA antibodies are present in 3-21.4% of SLE patients (Boey et al., 1988; Sakamoto et al., 1989; Swaak et al., 1990).

Autoantibodies as Markers Autoantibody profiles are important clinically in distinguishing SLE from many of the other autoimmune diseases, such as, scleroderma, Sjögren's syndrome (SS), mixed connective tissue disease (MCTD), and dermatomyositis/polymyositis. Certain antibodies, including anti-Sm and anti-dsDNA antibodies, have been shown to be specific diagnostic markers for SLE , whereas others, such as anti-ssDNA and anti-Ro antibodies, are only minimally helpful in narrowing the differential diagnosis.

Anti-Sm antibodies are generally considered pathognomonic for SLE (Notman et al., 1975) and are part of the American Rheumatology Association (ARA) 1982 revised criteria (Tan et al., 1982). Furthermore, a high titer of antibodies to dsDNA is considered a marker for SLE and is rarely present in other diseases (reviewed in Tan 1993; and Craft and Hardin, 1992). Whereas the sensitivity of anti-Sm antibodies is only approximately 30% for SLE, the majority of patients with active lupus have anti-dsDNA reactivity with moderate to high titers (Christian and Elkon, 1986). Anti-P antibodies have also been reported as fairly specific to SLE. Bonfa and Elkon found anti-P antibodies in 17 out of 20 (85%) SLE patients versus 0 out of 34 non-SLE patients (Bonfa and Elkon, 1986).

The other major antibody specificities of SLE are not commonly reported as being as specific for SLE. For example, anti-U1 RNP antibodies are also found in (and actually necessary for a diagnosis of) MCTD (reviewed in Tan 1989; and Craft and Hardin, 1992; and Craft et al., 1988) and, rarely, in rheumatoid arthritis, Sjögren's syndrome, scleroderma,

and polymyositis (reviewed in Craft and Hardin, 1992). Anti-U2 RNP antibodies have been described in patients with MCTD, psoriasis, scleroderma-polymyositis overlap syndrome, other overlap syndromes often including myositis, and patients without an identified disease (reviewed in Craft et al., 1988). Anti-histone antibodies are found additionally in juvenile rheumatoid arthritis, rheumatoid arthritis, and in high titer are characteristic of patients with drug-induced lupus (reviewed in Craft and Hardin, 1992). Anti-ssDNA is present in patients with other rheumatic diseases and in patients with nonrheumatic diseases, most commonly chronic infection (reviewed in Tan 1993).

In addition to SLE, anti-Ro and anti-La autoantibodies are found in the majority (approximately 70% and 45-60%, respectively) of patients with Sjögren's syndrome (reviewed in Tan 1993; Maddison et al., 1985). Ro and La specificities are also associated with polymyositis, scleroderma and rheumatoid arthritis (reviewed in Craft and Hardin, 1992). Anti-La antibodies are also detected in patients with primary biliary cirrhosis and patients without a clinical diagnosis (reviewed in Craft and Hardin, 1992). Anti-Ku antibodies are seen in patients with scleroderma and MCTD (Reeves 1985), and anti-Ki antibodies are found in patients with MCTD, primary Sjögren's syndrome, and rheumatoid arthritis, in addition to SLE (Sakamoto et al., 1989).

Autoantibodies as Monitors of Disease Activity

Autoantibodies have also been used to monitor disease activity. It has most clearly been shown that the titer of anti-dsDNA antibodies can correlate with SLE disease activity. Swaak, Tan, and others have noted a decrease in anti-dsDNA antibodies that correlates with clinical disease exacerbations, compatible with immune-complex formation (reviewed in Christian and Elkon, 1986). Other patients with active SLE have been noted to have titers magnitudes higher than normal controls and patients with inactive SLE (reviewed in Christian and Elkon, 1986). In a study of patients with connective tissue diseases, Houtman et al. correlated anti-nRNP/Sm levels with disease activity (Houtman et al., 1985). Boey and Tan studied 94 SLE patients and found that patients with anti-Sm antibodies were more likely to have active lupus (Boey et al., 1988). Scopelitis et al., in a small study, suggested that anti-Ro titers also correlate with disease activity (Scopelitis et al., 1980). Given that autoantibody titers fluctuate, some studies of SLE autoantibody profiles have used disease activity as a selection criterion including only those sera of patients with a disease exacerbation (Swaak et al., 1990).

Role of Autoantibodies in the Pathogenesis of Disease

In addition to their role as markers of disease and disease activity, autoantibodies in SLE have been shown to play a direct role in tissue damage. There is compelling evidence that dsDNA-antibody complexes are involved in the pathogenesis of renal disease in many SLE patients by mediating immune complex injury (Maddison et al., 1985) and can be a risk

factor for nephritis (reviewed in Craft and Hardin, 1992). Glomerular eluants have been shown to have more than thousand-fold concentrations of anti-dsDNA relative to the sera of the same patient (Christian and Elkon, 1986). Additionally, ssDNA is present in immune complexes in the glomeruli of patients with SLE (Maddison et al., 1985). Further studies have shown that the ability of anti-dsDNA antibodies to fix complement determines the pathogenicity (reviewed in Craft and Hardin, 1992).

Evidence exists that implies that anti-Ro antibodies may be directly involved in the pathogenesis of neonatal SLE, correlating with both an increased risk of neonatal skin rash and congenital complete heart block (Maddison et al., 1985). Interestingly, neonatal lupus is characterized by anti-Ro and anti-La antibodies in both the mother and the child, with the neonatal skin lesions disappearing together with the antibodies which were transferred transplacentally (Reichlin, 1985). Anti-Ro antibodies cause neonatal heart block by directly affecting the conduction system (Buyon 1992). In patients with subacute cutaneous lupus erythematosus (SCLE), studies have suggested that anti-Ro antibodies may bind to the Ro antigen on keratinocytes, contributing to the skin pathology characteristic of this disease (reviewed in Craft and Hardin, 1992). Human keratinocytes bind anti-Ro and anti-La antibodies if cultured in the presence of estradiol, and UV light has been shown to induce anti-Ro antibody binding to keratinocytes (reviewed in Tsokos 1992).

Correlation of Autoantibodies with Clinical Manifestations

In addition to these studies showing a direct pathologic role for antibodies, many past studies have examined the clinical significance of

autoantibody profiles in SLE patients by searching for associations between autoantibodies and clinical manifestations. Many investigators have studied a single antibody and tried to relate it to special clinical features. Others have realized that a particular autoantibody specificity does not stand alone and have tried relating antibody patterns to clinical features. Furthermore, certain antibodies and/or antibody combinations have often been proposed to be markers for particular subsets of patients. Associations previously reported in the literature are discussed below and shown in Table 2. Unfortunately, many reported associations do not duplicate when tested by other investigators on different populations and/or with different assays.

dsDNA One of the most widely recognized associations is that of anti-dsDNA, hypocomplementemia, and nephritis (reviewed in Tan 1989; and Craft and Hardin, 1992; Swaak et al., 1990). Swaak and colleagues looked at autoantibody profiles in a group of SLE patients in the Netherlands and found that in addition to anti-dsDNA antibodies correlating positively with nephritis, they correlate negatively with CNS manifestations (Swaak et al., 1990); and Thompson et al., in 1993, found an association between anti-dsDNA antibodies and hematologic disease and/or a malar rash (however these are not widely observed associations).

Histone In SLE patients, one study associated anti-histone antibodies with photosensitivity (reviewed in Tan 1989; and Swaak et al., 1990). More widely accepted, anti-histone antibodies, in the absence of anti-dsDNA, anti-Sm and other autoantibodies, are characteristic of drug-induced lupus. Interestingly, the pattern of anti-histone specificity varies depending if they

are drug-induced and if so, which drug caused the antibody response (Christian and Elkon, 1986). For example, procainamide-induced anti-histone antibodies are targeted to the H2A/H2B complex, whereas, hydralazine-induced anti-histone antibodies are IgM antibodies targeted against histones H3 and H2A (Christian and Elkon, 1986).

Ro and/or La Maddison and Reichlin, and others, have associated anti-Ro antibodies with severe photosensitive dermatitis. (As mentioned above, anti-Ro antibodies are believed to have a direct pathological affect on keratinocytes.) Some of these dermatitis patients have been classified into a subset of lupus designated subacute cutaneous lupus erythematosus (SCLE). Patients with subacute cutaneous lupus are highly photosensitive and have prominent skin lesions, characterized as a nonscarring dermatitis, in the setting of less other organ involvement. (reviewed in Maddison et al., 1985)

Maddison and Reichlin associated anti-Ro positive, anti-La negative, sera with lupus nephritis (Maddison et al., 1985). However, in this particular study 77% of patients positive only for anti-Ro antibodies also had anti-dsDNA antibodies which, as stated above, are generally agreed to be involved in the pathogenesis of renal disease in lupus (Maddison et al., 1985). In a retrospective study of SLE patients by Maddison et al. (reviewed in Reichlin 1985), anti-Ro antibodies were also associated with an increased frequency of rheumatoid factor positivity (Bell and Maddison, 1980) and coexistent Sjögren's syndrome or keratoconjunctivitis sicca (Reichlin 1985). Anti-Ro antibodies have also been associated with vasculitis (Reichlin 1985), hepatitis (reviewed in Craft and Hardin, 1992), decreased frequency of

Raynaud's (Bell and Maddison, 1980) and non-thrombocytopenic purpura (Reichlin 1985); however, these associations have not been confirmed.

Hamilton et al. studied antibodies to Ro, La, and Sm/nRNP in a group of SLE patients and identified two Ro autoantibody subgroups, associating the anti-Ro only group with HLA alleles DR2 and DQw1, and the anti-Ro and La subgroup with HLA alleles D8, DR3, DRw52, DQw2, older age at disease onset, sicca complex and less renal pathology (Hamilton et al., 1988). Anti-La antibodies are often associated with the sicca syndrome (reviewed in Craft and Hardin, 1992).

Sm Anti-Sm antibodies are found in a higher frequency in blacks than whites (Arnett et al., 1988; Ward and Studenski, 1990) and have been associated with decreased frequency of CNS disease manifestations and nephritis (Swaak et al., 1990) (although this may be assay-dependent; reviewed in Craft and Hardin, 1992) and, in one study, an increased frequency of malar rash and hematologic pathology (Thompson et al., 1993).

U1 snRNP Anti-U1 antibodies have also been associated with black race (Arnett et al., 1988). A study by Bell and Maddison looked at 64 patients and associated anti-U1 RNP with a lower frequency of serositis, renal disease, and Raynaud's phenomenon (Bell and Maddison, 1980). Swaak et al., however, looking at a Netherlands' population found anti-U1 RNP correlated with an increased frequency of pleuropericarditis and CNS manifestations (Swaak et al., 1990); and Thompson et al., in 1993, found an association with increased frequency of Raynaud's. Williamson et al correlated vasculitis with the presence of anti-U1 RNP (Williamson et al., 1983). These correlations have yet to be widely confirmed.

Ribosomal Proteins Bonfa and Elkon, in 1986, associated anti-ribosome P antibodies with lupus psychosis using Western blotting technique. In 1990, Swaak suggested that these antibodies are also associated with sicca syndrome.

Ki Tojo et al. suggested an association between anti-Ki antibodies and arthritis, pericarditis, fever, and pulmonary hypertension. Furthermore, an association between anti-Ki antibodies and a higher prevalence of CNS involvement has been suggested (Bernstein et al., 1984; Sakamoto et al., 1989; reviewed in Swaak et al., 1990).

PCNA Anti-PCNA antibodies have not been associated with any particular clinical symptoms (reviewed in Craft and Hardin, 1992).

Autoantibody Sets

The mutual occurrence of specific antibodies in sets has been evident to investigators since the first autoantibodies were reported. In 1973, Mattioli and Reichlin wrote a paper entitled "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" in which they discuss the mutual occurrence of Sm and U1 RNP autoantibodies. Since then, the occurrence of common autoantibodies in sets has been repeatedly confirmed. In addition to anti-Sm antibodies almost always accompanying anti-U1 RNP antibodies, anti-dsDNA antibodies often accompany anti-histone antibodies, and anti-La

antibodies/SS-B antibodies are almost always associated with anti-Ro antibodies (reviewed in Hardin 1986). Other sets identified include that antibodies to histones H1 and H2B are almost always found together (reviewed in Hardin 1986) and that anti-U2 RNP antibodies are associated with anti-U1 RNP antibodies (Craft et al., 1988). Furthermore, these antibodies tend to occur in an ordered sequence (i.e., U1 RNP occurs before Sm and Ro before La) (reviewed in Hardin 1986).

These sets are characterized by targeting particles that are a part of the same macromolecular structure, and as discussed by Hardin in 1986, "the most prominently recognized autoantigens reside on 3 types of nucleoprotein particles: the nucleosome, the U1 snRNP and the Ro scRNP [small cytoplasmic ribonucleoprotein] [sic]." These observations led Hardin and Tan to hypothesize independently that the immune response in SLE targets macromolecular particles (Hardin 1986, reviewed in Theofilopoulos 1995).

Etiology of SLE

The etiology of the autoimmune response in systemic lupus erythematosus remains unclear. Epidemiological studies have suggested that susceptibility to lupus is multifactorial with investigations suggesting genetic, hormonal and environmental components to SLE (reviewed in Sinha et al., 1990). Evidence supporting a genetic component includes that lupus is associated with the major histocompatibility complex (MHC) molecules HLA-B8, DR2, DR3, and DQw1 (Maddison et al., 1985). (However, monozygotic twin pair studies show a concordance less than 100% indicating that MHC genes are not the sole factor in determining

lupus) (reviewed in Sinha et al., 1990). A 9:1 female to male ratio in patients with SLE is well documented and strongly suggests a hormonal component to SLE. Other evidence for the role of sex hormones includes that testosterone enhances suppressor cell activity; estrogens have a stimulatory effect on B cells and a suppressive effect on regulatory cell activity; and lymphocytes respond with increased activity to pokeweed mitogen in the presence of estradiol and with decreased activity in the presence of testosterone (reviewed in Tsokos 1992). Current possibilities for the genesis of SLE, which will be briefly reviewed below, include: the particle hypothesis, the modified self hypothesis, the molecular mimicry model, polyclonal B and/or T cell activation, the release of anatomically sequestered antigens, the "cryptic self" hypothesis, the self-ignorance hypothesis, errors in B and/or T cell tolerance, and defects in immunoregulation (reviewed in Theofilopoulos 1995).

The Particle Hypothesis As stated above, the autoantibodies of lupus patients commonly occur in sets (DNA/histone, Sm/U1 RNP, and Ro/La). These sets are characterized by targeting particles of common macromolecular structures: the nucleosome, the spliceosome and the Ro ribonucleoprotein. Such observations led to the hypothesis that the immune response in SLE targets macromolecular particles (Hardin 1986). The particle hypothesis of autoimmunization, proposed independently by Tan and Hardin, suggests that the total autoimmunogenic repertoire of lupus is localized on a limited number of subcellular particles (Hardin 1986, reviewed in Theofilpoulos 1995).

Modified Self Model In 1986, Hardin suggested that an inciting agent may structurally alter selected macromolecules, making them antigenic (Hardin 1986). In support of this modified self model, patients with drug-induced lupus target the same histone epitopes as patients with spontaneous lupus (Grayzel et al., 1991).

Molecular Mimicry Model Infection has been shown to precede the onset of lupus which, together with the genetic predisposition, supports the molecular mimicry model. This model suggests that an exogenous agent bearing an epitope identical or similar to a host protein triggers an autoantibody response that may diversify (via cognate T cell - B cell interactions) to include autoantibodies to other epitopes on the inciting autoantigen or to an epitope on proteins of a multiprotein particle. Many studies support this theory.

Lerner et al., in 1981, observed that certain Epstein-Barr encoded RNAs (EBER) are specifically precipitated by anti-La antibodies supporting the hypothesis that the immune response to La is triggered by binding of host proteins bearing these antigenic determinants to products of viral infection, the resultant complex being immunogenic (Lerner et al., 1981). Also, Chan et al. showed similarities between La and the adenovirus 72 kD DNA binding protein (Chan et al., 1986). Grayzel et al. studied the sera of polyvalent pneumococcal polysaccharide vaccinated nonautoimmune individuals showing a rise in anti-pneumococcal antibodies targeting DNA (anti-dsDNA associated idiotype) (Grayzel et al., 1991). Elkon et al. showed that the properties of the ribosomal protein autoantigen are similar to those of foreign protein antigens (Elkon et al., 1988).

Polyclonal B Cell Activation (PBCA) These above theories alone do not explain the observation that autoantibodies in the same patient may target proteins from separate intracellular particles (a significant number of patients have antibodies to cytoplasmic, cell surface and nuclear antigens) (Grayzel et al., 1991). The great diversity of autoantibodies in SLE is quoted as being one of the major pieces of evidence supporting polyclonal B cell activation as a primary feature in the pathophysiology of the lupus immune response (reviewed in Hardin 1986). Earlier models of PBCA suggested that it was secondary to intrinsic B cell hyperactivity or suppressor T cell deficiencies (reviewed in Hardin 1986). Current findings which support PBCA in SLE include: the number of B cells that secrete immunoglobulin is increased in lupus; the number of spontaneously activated B cells correlates with disease activity, serum-DNA binding and low levels of serum C3; and bone marrow from patients with SLE contains large numbers of B cells autonomously secreting immunoglobulin (reviewed in Tsokos 1992).

Polyclonal T Cell activation (PTCA) On the other hand, a diversity of autoantibodies could also be secondary to intrinsic T-helper (T_h) cell hyperactivity - PTCA. In various murine models, T cell contact is required for B cell production of polyclonal immunoglobulins (Fatenejad et al., 1993). Accordingly, anti-CD4 (a surface marker of T_h cells) monoclonal antibodies prevent polyclonal Ig synthesis, and autoantigen-specific T cells are necessary for anti-dsDNA production (reviewed in Craft 1994 unpublished). Studies on human lupus patients have yielded isolated autoantigen-specific T cells (reviewed in Tsokos 1992). In 1993, a small clinical trial by Tokuda et

al. suggested that cyclosporin A (CsA), an inhibitor of T_h and cytotoxic cell activation, reduces the disease activity of SLE (Tokuda 1994).

Release of Anatomically Sequestered Antigens Interestingly, many antigens in lupus are intracellular and are in "privileged" sites that are normally inaccessible to circulating autoantibody. Hardin suggested that these intracellular particles may be protected by lower levels of tolerance (Hardin 1989). It is unclear how such intracellular antigens become involved in pathogenesis; however, studies have shown that intracellular molecules can escape autolytic degradation and be released into the extracellular environment. These molecules are then capable of being targeted by preexisting circulating antibody. This is seen in lupus when anti-dsDNA antibodies complex with DNA and initiate pathology. Furthermore, other normally intracellular antigens have been located extracellularly. Ro and Sm have been identified on keratinocytes and Sm is expressed on the cell surface in the kidney in some patients. (reviewed in Tan 1991)

"Cryptic Self" Hypothesis As explained in a recent review by Theofilopoulos (1995), this theory is based on the concept that MHC molecules usually process and present "self-determinant" proteins which constitute the dominant self. The immune system is normally tolerant to these self-proteins, but there are poorly displayed cryptic determinants which do not induce tolerance. Cryptic epitopes, generated by aberrant antigen processing of self or foreign polypeptides, may thus activate autoreactive cells and promote autoantibody production. For example,

cytochrome C peptide has been shown to contain a "cryptic" peptide (Mamula 1993).

Self-ignorance Hypothesis The self-ignorance hypothesis suggests that T cells specific for extrathymic antigens undergo anergy because of the absence of appropriate "costimulatory" factors. A popular model for this theory is that a virus, such as coxsackievirus or mumps, upregulates MHC and costimulatory factors, and contributes to β islet cell destruction in diabetes mellitus (Hou et al., 1993; Gerling et al., 1991; Loria et al., 1984; Parkkonen et al., 1992; Szopa et al., 1993; Vuorinen et al., 1992).

T and/or B Cell Tolerance Defect This theory suggests that in the normal host, autoreactive T and/or B cells are present but by some mechanism are tolerant. A defect in this mechanism could hypothetically result in a polyclonal autoantibody response. Various evidence has been accumulated to support this theory. For example, SLE-prone lpr mice are defective in the Fas apoptosis gene and have activated autoreactive T and B cells due to a defect in peripheral programmed cell death. (reviewed in Theofilopoulos 1995)

Immunoregulatory Disturbances Certain T cell regulatory subsets have been suggested to induce or inhibit disease development. Specifically,

(A) Defects of T-suppressor lymphocyte function may be involved in the pathogenesis of SLE. In support of this theory, the absolute number of T lymphocytes in SLE patients is decreased whereas peripheral B lymphocytes are present in normal number. More specifically, SLE patients have a decrease in the suppressor/cytotoxic lymphocyte

subpopulation, as defined by the presence of cell surface markers. Furthermore, Tar cells, which are considered to be precursors of suppressor/effector cells, are decreased in SLE. Low activity of natural killer cells, which are known to suppress B cell function and kill virus-infected cells, is associated with SLE disease activity. Also, some studies have shown that concanavalin A-induced suppressor cell function in patients with SLE is deficient. Deficient suppressor cell activity has been shown to correlate with SLE disease activity, serum DNA binding and low serum C3 levels. Epstein-Barr virus associated suppressor/cytotoxic cell function in EBV seropositive SLE patients has also been shown to be defective, supporting a secondary infectious component in addition to a primary defect in immunoregulation. (reviewed in Tsokos 1992)

(B) Increased T-helper activity has also been considered a possible etiologic theory. Evidence for this theory comes from several sources. First, patients with active SLE have been shown to have increased expression of DR antigens on the surface of their T cells. DR^+ cells provide help to autologous B cells to secrete immunoglobulin. Furthermore, in several lupus patients and some murine models of lupus, T cell subpopulations which provide help to B cells to secrete immunoglobulin have been isolated. For example, the $CD3^+CD4^-CD8^-$ subpopulation in SLE patients with active disease have been shown to provide help to autologous B cells to secrete anti-dsDNA antibodies, whereas normal controls failed to do so. (reviewed in Tsokos 1992)

Problems with the theory of a defect in immune regulatory cells include that anti-T-lymphocyte antibodies have been noted in lupus and that anti-dsDNA antibodies have been found to bind surface structures of normal human T cells. Consequently, the decreased number of

lymphocytes in SLE may be a result and not a cause of lupus. (reviewed in Tsokos 1992)

The above etiologic theories can be grossly divided into two main categories: those that support a global defect in immune tolerance versus those that support a single immunogen-guided response. Because a limited number of autoantigens appear targeted in SLE, autoantibody profiles can be examined hoping to support one main theory. A simple way of addressing this question is to ask how many different immunogens a single patient's serum targets. Two studies in the past have addressed the average number of antibody specificities per patient: in 1975, Notman and Tan found nine SLE patients had three or four individual specificities (Notman et al., 1975). This was confirmed by Boey and Tan in 1988 who found an average of 2.9 antibodies per SLE patient. The presence of more than one autoantibody per patient could be used to support a global defect in tolerance; however, given the hypothesis by Hardin and Tan that the autoimmune response targets particles (e.g., the nucleosome, the Ro/La RNP particle, the spliceosome), it may be more relevant to examine the number of autoantibody sets, not individual specificities, per patient. This question has not been previously addressed in the literature. The present study addressed this question by immunologically studying a group of SLE patients followed at Yale University and the surrounding New Haven area, as well as reanalyzing the autoantibody profiles previously reported in the literature. The sensitive and specific methods of ELISA and immunoprecipitation were used to determine the specificities of each patient's serum. Since this patient population was previously unstudied, the prevalence of defined autoantibodies are reported, and

autoantibody/clinical correlations were examined to study if associations found in other populations could be confirmed.

Table 1
Common antigens in systemic lupus erythematosus.

Cellular antigen	Characteristics	Frequency in SLE	Reference
dsDNA	Nucleic acid	40	Tan 1989
ssDNA	Nucleic acid	70	Tan 1989
Histones	H1, H2A, H2B, H3-4 proteins	30-70	Swaak et al., 1990 Tan 1989
Sm	mRNA processing U1, U2, U4-6 snRNA B,B', D, E proteins	15-30	Swaak et al., 1990 Tan 1989
U1 RNP	mRNA processing U1 snRNA 70 kD, A, C proteins	32	Boey et al., 1988
Ro/SSA	RNA processing 60kD, 52kD proteins Y1-Y5 RNA	25-35	Tan 1989 Reichlin 1985
La/SSB	RNA polymerase III termination complex 46kD, 48kD phosphoproteins RNA	15	Tan 1989
Ribosomal P proteins	Protein translation 38kD, 16kD, 15kD phosphoproteins	10-15	Bonfa and Elkon, 1986 Christian and Elkon, 1986 Elkon et al., 1988
PCNA/cyclin	DNA replication 36kD protein	3/7.9-21.4	Boey et al., 1988 Swaak et al., 1990 Sakamoto et al., 1989
Ku	86kD, 66kD nuclear protein	5-10	Boey et al., 1988 Tan 1989 Sakamoto et al., 1989
SL/Ki	32kD protein	6.3-21	Boey et al., 1988 Bernstein et al., 1984 Reichlin 1985 Swaak et al., 1990

* adapted from a review by Tan

Table 2
Clinical correlations found in autoantibody profile studies in the literature.

Antibody	Clinical/Lab Association	References
dsDNA	Nephritis, negative CNS, malar rash, renal, hematologic, hypocomplementemia	Thompson et al., 1993 Swaak et al., 1990
Histone	Photosensitivity, drug induced lupus	Thompson et al., 1993 Swaak et al., 1990
Ro	HLA-B8/DRw3, skin rash, RF positivity Congenital heart block, neonatal lupus photosensitivity, Sjögren's syndrome subacute cutaneous lupus, hepatitis vasculitis, thrombocytopenic purpura HLA-DR2/DQw1	Bell and Maddison,198(Maddison et al., 1985 Thompson et al., 1993 Craft and Hardin, 1992 Thompson et al., 1993 Reichlin, 1985 Hamilton et al., 1988
La	CNS, rashes, photosensitivity, Sicca syndrome, negative nephritis, HLA-D8/DR3/DRw52/DQw2	Craft and Hardin, 1992 Maddison et al., 1985 Hamilton et al., 1988 Thompson et al., 1993 Swaak et al., 1990
Sm	Negative CNS or nephritis, malar rash renal, hematologic, hypocomplementemia	Thompson et al., 1993 Swaak et al., 1990
U1RNP	Vasculitis CNS, pleuropericarditis negative serositis, renal disease, and Raynaud's increased Raynaud's, hematologic	Williamson et al., 1983 Swaak et al., 1990 Bell and Maddison,198(Thompson et al., 1993
Ki	CNS, arthritis, pericarditis, pulmonary HTN, fever	Sakamoto et al.,1989,3'
Ribosomal P proteins	Sicca syndrome, lupus psychosis	Swaak et al., 1990 Bonfa and Elkon, 1986
Other		
Ro and U1RNP"-"	Less Raynaud's	Bell and Maddison,198(
dsDNA &/or Sm	Proteinuria, renal casts, leukopenia, lymphopenia, thrombocytopenia, hypocomplementemia, malar rash, increased prevalence and severity of clinical manifestations	Thompson et al., 1993

PATIENTS AND METHODS

Sera. Serum samples from 68 patients with systemic lupus erythematosus were obtained from the Division of Rheumatology, Department of Medicine, Yale University. All patients were diagnosed with SLE based on fulfilling at least four of 11 of the American Rheumatology Association's 1982 Revised Criteria for the Classification of SLE.

Sera from 11 lab technicians were used as normal controls. Prototype sera containing antibodies to Ro, La, U1 RNP , Sm, dsDNA, histones, Ku, Ki, PCNA, and ribosomes were previously obtained by Dr. Joseph Craft. Six negative human plasma controls and six standard human plasma controls containing antibodies to Ro, La, U1 RNP, Sm, dsDNA, and histones were obtained from Apotex Scientific, Inc.

ELISAs. Kits were obtained from Apotex Scientific, Inc., and sera were tested as per protocol outlined by Apotex. Patient and control sera were diluted (1:500 for Ro, La, U1, and Sm; and 1:100 for DNA and histones) in phosphate, BSA and 0.5% sodium azide buffer and incubated with antigen for 60 minutes. Coated wells were washed with borate and 0.8% sodium azide buffer three times, and antigen-antibody product was labeled with alkaline phosphatase labeled anti-human IgG murine monoclonal antibody for 30 minutes. After three washes with borate buffer, labeled antigen-antibody complexes were developed with Mg^{2+}/phenolphthalein monophosphate substrate for 30 minutes. The alkaline phosphatase enzyme reaction was stopped with EDTA solution and the absorbance was read at 550nm (Titertek Multiskan model 310 spectrometer).

Preparation of radiolabeled cell extract. HeLa cell extract was prepared as previously described (Craft and Hardin 1992). HeLa cells were radiolabeled for 8-14 hours with ^{35}S-methionine (5uCi/ml of cells; ICN Biomedical, Irvine, CA), collected by centrifugation for 10 minutes at 1,000*g*, washed in 10-12 pellet volumes of Tris-buffered saline (TBS; 10mM Tris-Cl, pH 7.4, 150mM NaCl), and resuspended in immunoprecipitation buffer (IPP; 10mM Tris-Cl, pH 8.0, 500mM NaCl, 0.1% Nonidet P-

40). Resuspended labeled cells were sonicated 3 times each for 30 seconds with a Branson sonifier at setting 3, centrifuged for 15 minutes at 15,000*g* 4° C to remove cellular debris, and the supernatant was collected.

Immunoprecipitation of radiolabeled cell extracts. Immunoprecipitation of radiolabeled cell extract was performed as previously described (Craft and Hardin 1992). Five µl patient or 1-5 µl control sera and 2 mg protein A-Sepharose CL-4B (Pharmacia, Piscataway, NJ) were combined in 400 ul IPP buffer by end-over-end rotation (Labquake Shaker; Lab Industries, Berkeley, CA) for two hours at 4° C. After three washes with IPP buffer, the antibody-coated Sepharose beads were resuspended in 400 µl IPP buffer and combined with 50-100 µl ^{35}S labeled cell extract by end-over-end rotation for 2 hours at 4° C. Antibody-protein complexes were washed three times with cold IPP buffer, resuspended in 3 to 4 pellet volumes of SDS-sample buffer (2% SDS, 10% glycerol, 0.5M Tris Cl/0.4% SDS, pH 6.8, mercaptoethanol, 0.005% bromphenol blue); and after vortexing and heating at >95° C for 5 minutes, the proteins were fractionated on SDS polyacrylamide gels. The gels were enhanced with 0.5M sodium salicylate, dried and analyzed by autoradiography. Results were based on comparison with prototypic sera (Figures 1, 2 and 3).

Meta-analysis. A literature search was carried out and previously reported autoantibody profiles of SLE patients were analyzed. The minimal average number of autoantibody sets per patient was calculated based on the reported data. In studies assaying for only several autoantibodies, the calculated average number of autoantibody sets per patient may grossly underestimate the actual number of sets.

Clinical Evaluation. Patients were evaluated by retrospective clinical chart review (raw data collected largely by Dr. Robert McClean, Rheumatology Fellow at the Yale University School of Medicine). All 68 patients included in the study were diagnosed with SLE according to the ARA's 1982 Revised Criteria. Clinical information on patients was cumulative and not necessarily obtained solely at the time sera were drawn.

Dermatologic disease was diagnosed by the presence of malar rash, photosensitivity, alopecia, mucous membrane ulcers, discoid rash, livedo reticularis, subcutaneous nodules, and/or clinically reported symptoms of sicca syndrome. Serositis was diagnosed clinically and/or radiologically. Musculoskeletal disease was defined by arthritis involving two or more peripheral joints, clinically reported myalgias or arthralgias, and/or myositis accompanied by elevated muscles enzymes. Neurological disease included clinical psychosis, peripheral neuropathy, and/or seizures in the absence of drugs or other known metabolic causes. Vascular disease was defined by clinically reported Raynaud's, vasculitis, cutaneous vasculitis, deep venous thrombosis by Doppler ultrasound, and/or digital or leg ulcerations thought secondary to vascular pathology. Renal disease was diagnosed histologically, on the basis of persistent proteinuria (>0.5gm/day), and/or cellular cast(s) on urinalysis. Hematologic disorders were defined by hemolytic anemia (documented by positive Coombs and/or reticulocytosis), leukopenia ($<4,000/mm^3$), lymphopenia ($<1,500/mm^3$), and/or thrombocytopenia ($<100,000/mm^3$). Hypocomplementemia and rheumatoid factor positivity were defined according to individual laboratory guidelines.

Sample size was determined by a nomagram, using the standard difference (SD) of the samples and a statistical power of 80% ($\beta < 0.20$). Standard difference was determined as

$$\frac{p_1 - p_2}{\mathrm{SQRT}[p_m(1-p_m)]}$$

where p_1 and p_2 are the expected proportions of events in the experimental and control groups, respectively, and p_m is the mean of the proportions $((p_1 + p_2)/2)$. The number of experimental and control specimens required is equal to half of the resulting sample size (n).

The experimental and control specimens were not equivalent in number; therefore, a correction for power was made. The method of estimating the sample size for a given power estimation used was:

$$N = \frac{N'(1 + k)^2}{4k}$$

where k = the ratio of experimental specimens to control specimens and N' = the original sample size (n) required as determined by nomogram.

Given 68 experimental patients and 12 control patients, k = 5.7. The adjusted sample size is (rearranging the above equation):

$$\frac{N\,(4)\,k}{(1 + k)2}$$

or 41. By nomagram, this gives a SD of approximately 0.875 for a significance of 95% ($\alpha = 0.05$).

By empirically testing p_I's, the SD equals 0.84 for a prevalence of 30%. Hence, 80 patients containing 12 control patients supplied enough specimens to determine statistical significance at 95% confidence and 80% power, as long as the prevalence of the tested autoantibody or clinical characteristic was 30%. (If 68 negative controls would have been run, a prevalence of 10% would have been statistically significant.)

Clinical/autoantibody associations were determined using Chi-square analysis with Yates' correction. A resulting P-value < 0.05 was considered significant. Clinical manifestations and antibodies with a frequency > 10% in the study population were analyzed, however only the tested entities with a frequency of > 30% have a statistical power of 80%.

RESULTS

The Study Population Sixty-eight patients fulfilling the ARA Revised Criteria for systemic lupus erythematosus were studied (Table 3). The female to male patient ratio was 9:1. The white to black patient ratio was 3:1. The mean age at disease onset was 30.2 +/- 13.9 years. One quarter of the patients reported a first degree relative as having either rheumatoid arthritis (RA) or SLE. The mean duration of disease at the time the sera samples were drawn was 8.2 years. The mean duration of disease at the time of the chart review was 10.9 years.

Clinical Aspects Initial clinical factors are shown in Table 4. Possible precipitating events identified include pregnancy in nine patients and infection in two patients. The initial clinical signs/symptoms reported include, in decreasing order of frequency: arthralgias, arthritis, rash, ITP, fatigue, serositis, Raynaud's, extremity weakness, alopecia, photosensitivity, subcutaneous nodules, myalgias, aseptic meningitis, cutaneous vasculitis, proteinuria, hemolytic anemia, dyspnea, and chest pain. Fourteen patients had greater than or equal to two initial signs or symptoms.

Cumulative frequencies of the individual clinical manifestations of SLE in the study population are shown in Table 5. Chi-square analysis with Yates' correction was used to study associations between autoantibodies and SLE clinical manifestations. Those clinical manifestations and antibodies of > 10% frequency in the study population were considered, including: dermatologic disease as a whole (malar rash, alopecia, photosensitivity, mucous membrane ulcers, discoid rash, livedo reticularis,

sicca syndrome, and/or subcutaneous nodules), malar rash, alopecia, photosensitivity, mucous membrane ulcers, discoid rash, serositis as a whole (pleuritis and/or pericarditis), musculoskeletal disease as a whole (arthritis, arthralgias, myalgias, and/or myositis), arthritis, neurological manifestations of disease as a whole (psychosis, peripheral neuropathy, and/or seizures), Raynaud's, vasculitis, cutaneous vasculitis, history of a thrombotic event, renal disease as a whole (proteinuria, RBC casts in urine, and/or renal failure), proteinuria, RBC casts in urine, hematologic disease as a whole (leukopenia, hemolytic anemia, and/or thrombocytopenia), leukopenia, hemolytic anemia, thrombocytopenia, hypocomplementemia, rheumatoid factor positivity, and corticosteroid treatment at the time of phlebotomy.

The following entities were not studied because of either a lack of statistical power of 80% secondary to a frequency less than 10% or a subjective lack of utility: livedo reticularis, sicca syndrome, subcutaneous nodules, pericarditis, myalgias, myositis, seizures, vascular disease as a whole, digital ulcerations, leg ulcerations, renal failure, lymphopenia, VDRL false positivity, headache, and thyroiditis.

The associations found are shown in Table 5. A P value ≤ 0.05 for a confidence level $\geq 95\%$ was considered statistically significant. Notable findings by autoantibody follow. Anti-dsDNA antibodies were associated with serositis, pleuritis ($\beta > 0.20$), musculoskeletal disease manifestations, arthritis, renal disease, and hypocomplementemia. No association between dsDNA antibodies and CNS disease was found. Anti-histone antibodies were associated with discoid rash ($\beta > 0.20$), serositis, musculoskeletal disease manifestations, leukopenia, thrombocytopenia ($\beta > 0.20$), and hypocomplementemia. Anti-Sm antibodies were associated with

serositis and pleuritis ($\beta > 0.20$). No association between race and Sm was shown ($\beta > 0.20$). Anti-U1 RNP antibodies correlated with cutaneous disease, serositis, and pleuritis ($\beta > 0.20$). Anti-Ro antibodies were associated with neurological disease and hemolytic anemia. No correlation between anti-Ro antibodies and RF positivity was found. Anti-La antibodies were associated with RF positivity.

Forty-three percent of the study population were receiving corticosteroid treatment at the time of phlebotomy. Corticosteroid treatment correlated with autoantibodies to histones, dsDNA and/or Ro.

Inter-autoantibody associations were studied (Table 6). Anti-histone antibodies associated with anti-dsDNA antibodies, anti-U1 RNP antibodies, and weakly with anti-Ro antibodies. Anti-dsDNA antibodies were also associated with anti-U1 RNP antibodies and anti-Sm antibodies. Notably, anti-Sm antibodies did not statistically correlate with anti-U1 RNP antibodies, however, the chi-squared analysis is not statistically significant at an Sm frequency of 14.7%. The study did show that 6 of 10 Sm positive patients had the U1 RNP specificity as well. Anti-Ro and anti-La antibodies were statistically associated.

Autoantibodies Sera of the 68 SLE patients and 12 controls were studied by ELISA and immunoprecipitation for the following autoantibody specificities: dsDNA, histone, Sm, U1 RNP, Ro, La, rRNP, PCNA, Ku, Ki, and other autoantibodies (unidentified band(s) by immunoprecipitation). The following autoantibody sets were considered: dsDNA and/or histone, Sm and/or U1 RNP, Ro and/or La, rRNP, PCNA, Ku, Ki, and other (unidentified protein bands on SDS polyacrylamide gels after ^{35}S

immunoprecipitation). The results by patient number and frequency percent are shown in Tables 7 and 8.

Autoantibody frequency (%) results by ELISA include: anti-dsDNA 39.7%, anti-histone 51.5%, anti-Sm 14.7%, anti-U1 33.8%, anti-Ro 39.7%, and anti-La 14.7%. Autoantibody set results by ELISA include: anti-dsDNA and/or anti-histone 58.8%, anti-Sm and/or anti-U1 RNP 39.7%, and anti-Ro and/or anti La 41.2%.

Using ^{35}S immunoprecipitation, the following specificities in frequency percent resulted: anti-Ro 42.6% and anti-La 19.1%. By autoantibody set, the following frequencies were found: anti-Sm and/or anti-U1 RNP 39.7%, and anti-Ro and/or anti-La 44.1%. Protein bands on SDS polyacrylamide gels similar to those found in prototypic sera with specificities for ribosomal proteins, Ku, and Ki were found in the following percentages, respectively: 4.4%, 4.4%, and 2.9%. These frequencies are not sufficiently high for significant statistical analysis as stated previously. Fourteen (20.5%) patients had protein bands of unidentified specificities.

Eleven patients (16%) were autoantibody negative by both ELISA and immunoprecipitation. All positive control plasma samples were positive for respective specificities by ELISA. All negative control plasma and sera were autoantibody negative by ELISA. By immunoprecipitation, no negative control sera had autoantibodies to Sm/U1 RNP, Ro, La, rRNP, PCNA, Ku or Ki. Three normal control sera had protein bands of unidentified specificity.

The average number of antibodies and antibody sets per patient were determined as 2.34-2.37 and 1.72-1.74, respectively (Table 9). Considering only antibody positive patients, the average number of antibodies and antibody sets per patient were 2.79-2.82 and 2.01-2.07, respectively. The

distributions of the number of antibodies and antibody sets per patient are shown in Figures 4 and 5. Using ELISA and immunoprecipitation results: twelve patients each had one, two, or three autoantibodies; thirteen patients had four antibodies; five patients showed five antibodies; and two patients had six antibodies. By antibody set: 17 patients had one set, 19 patients had two sets, 14 patients had three sets, and five patients had four autoantibody sets. (Table 8)

The number of autoantibody sets by duration of disease at the time of phlebotomy was plotted (Figure 6). No correlation was shown.

Autoantibody profiles found in the literature are shown in Table 8. Data sets were analyzed for the minimal possible number of reported antibody sets per patient (Table 10). Twelve studies found at least greater than than one set per patient, three studies found at least greater than two sets per patient, and one study found at least greater than three sets per patient.

Table 3
Characteristics of the study group.

Total number of patients = 68			
Sex	Female	61	(90%)
	Male	7	(10%)
Race	White	42	(62%)
	Black	15	(22%)
	Hispanic	1	(1.5%)
	Unknown	10	(15%)
Mean age at disease onset (years) = 30.2 SD +/- 13.9			
Median age at disease onset (years) = 29.0			
Mean duration of disease at time of phlebotomy (years) = 8.2			
Mean duration of disease at time of chart review (years) = 10.9			
Positive family history = 18 (26%)			

Table 4
Initial Clinical Factors.

Possible precipitating event	Number of patients	
Pregnancy	9	
Infection	2	
First clinical sign/symptom		
arthralgias	19	
arthritis	13	
rash	6	(1 discoid)
ITP	4	
fatigue	4	
serositis	3	
Raynaud's	3	
extremity weakness	2	
alopecia	1	
photosensitivity	1	
subcutaneous nodules	1	
myalgias	1	
aseptic meningitis	1	
cutaneous vasculitis	1	
proteinuria	1	
anemia	1	
dyspnea	1	
chest pain	1	

Patients reporting ≥ 2 initial signs/symptoms = 14

Table 5
Clinical profile of SLE patient population.

Disease manifestation	No. with diagnosis	Frequency of occurrence (%)	Antibody association	P
Cutaneous	55	81%	U1 RNP	<.05
malar rash	34	50		
alopecia	28	41		
photosensitivity	27	40		
mucous membrane ulcers*	19	28		
discoid rash*	8	12	His	<.05
livedo reticularis*[1]	4	6		
sicca syndrome *[1]	2	3		
subcutaneous nodules *[1]	2	3		
Serositis	24	35	dsDNA	<.01
			Sm	<.01
			U1 RNP	<.05
			His	<.05
pleuritis*	18	27	dsDNA	<.01
			U1 RNP	<.05
			Sm	<.05
pericarditis*[1]	6	9		
Musculoskeletal	57	84	His	<.05
			dsDNA	<.05
arthritis	48	71	dsDNA	<.05
arthralgias[1]	27	40		
myalgias*[1]	6	9		
myositis*[1]	3	4		
Neurological manifestations	22	32	Ro	<.02
psychosis*	12	18		
peripheral neuro*	7	10		
seizures*[1]	2	3		
Vascular[1]	35	52		
Raynaud's	21	31		
vasculitis	21	31		
cutaneous vasculitis*	16	24		
thrombotic event*	7	10		
digital ulcerations*[1]	6	9		
leg ulcerations*[1]	1	2		
Renal	30	44	dsDNA	<.05
proteinuria	27	40		
RBC casts in urine	22	32		
renal failure*[1]	6	9		

Table 5 (continued)

Disease manifestation	No. with diagnosis	Frequency of occurrence (%)	Antibody association	P
Heme[1]	43	63%		
leukopenia <4K	25	37	His	<.05
hemolytic anemia*	20	30	Ro	<.02
thrombocytopenia * (<100K)	12	18	His	<.05
lymphopenia *[1] (<1.5K)	4	6		
Constitution symptoms[1]	40	59		
fatigue[1]	34	50		
fever[1]	21	31		
wt loss (>5 lb.)*[1]	8	12		
Other				
Hypocomplementemia			dsDNA His	<.01 <.02
low C3[1]	32	67		
low C4[1]	30	63		
Rheumatoid Factor * (out of 22 patients)	9	41	La	<.01
VDRL false "+" *[1] (out of 17 patients)	5	29		
HA *[1]	8	12		
thyroiditis *[1]	5	7		
Corticosteroid Tx	29	43	His dsDNA Ro	<.01 <.01 <.05

* $\beta > 0.20$

[1]not tested for autoantibody correlation

Table 6
Inter-autoantibody Associations.

Antibody	Associated Antibody	Chi-Squared	P
Histone	dsDNA	19.0	<.001
	U1 RNP	13.6	<.001
	Ro	3.86	<.05
dsDNA	Histone	as above	
	U1 RNP	6.58	<.02
	Sm	4.37	<.05
Sm	dsDNA	as above	
	U1 RNP	2.92*	<.1
U1 RNP	Histone	as above	
	dsDNA	as above	
	Sm	as above	
Ro	La	3.86	<.05
	Histone	as above	
La	Ro	as above	

*$\beta > 0.20$

Table 7
Autoantibody profiles (Part I).

Specificity	No. of pts with specificity		Frequency (%)	
	ELISA	IPP	ELISA	IPP
dsDNA	27		40%	
Histone	35		52	
dsDNA/Histone set	40		59	
Sm*	10		15	
U1 RNP	23		34	
Sm/U1 RNP set	27	27	40	40%
Ro	27	29	40	43
La*	10	13	15	19
Ro/La set	28	30	41	44
?rRNP*		3		4
?PCNA*		0		0
?Ku*		3		4
?Ki*		2		3
?Other		14		21
High Band (HB) A 170 kD		3		
HB B 125 kD		1		
HB C 100 kD		1		
HB D 98 kD		1		
HB E 97 kD		1		
HB F 78 kD		1		
HB G 75 kD		1		
HB I 70 kD		1		
Low Band (LB) A 43 kD		1		
LB B 35 kD		1		
LB C 17 kD		1		
Three bands		1		

*$\beta > 0.20$

Table 8
Autoantibody profiles (Part II).

Number of antibodies or set(s)	Number of patients ELISA	IPP	Frequency (%) of pts ELISA	IPP
1 antibody	14	12	21%	18%
2 antibodies	11	12	16	18
3 antibodies	13	12	19	18
4 antibodies	11	13	16	19
5 antibodies	5	5	7	7
6 antibodies	2	2	3	3
1 antibody set	18	17	27	25
2 antibody sets	20	19	29	28
3 antibody sets	13	14	19	21
4 antibody sets	5	5	7	7
Autoantibody neg. patients*	11	11	16	16

*$\beta > 0.20$

Table 9
Autoantibodies or antibody sets per patient.

	ELISA	IPP
Average number of antibodies/ patient	2.34	2.37
Average number of antibodies/autoantibody positive patient	2.79	2.82
Average number of antibody sets/patient	1.72	1.74
Average number of antibody sets/autoantibody positive pt.	2.05	2.07

* $\beta > 0.20$

Table 10
Autoantibody profiles in the literature.

Author	Autoantibody Frequency (%)							Antibody sets/pt
	dsDNA	His	Sm	U1RNP	Sm/U1	Ro	La	
This study								1.72
68 pts	40	52	15	34	40	40-43	15-20	
Arnett (1988)[2,3,4]								≥1.1
60 Black pts			25	40	52	58	20	
106 White pts			10	23	26	45	20	
Bell and Maddison (1980)[5]								≥1.08
64 White pts		61		22	25	2.5	25'	
Bernstein et al. (1984)[4]								
270 pts			7	23		24	8	
Boey et al. (1988)[6]								
94 Asian pts	43	81	26	32		63	12	≥2.09
9 psych pts	88	100	77	66				≥3.64
Bonfa and Elkon (1986)[4,7]								
rRNP "+" pts								
18 psych pts	44		33	33		50	11	≥2.27
14 nonpsych pts	50		36	36		36	0	≥2.22
Hamilton et al. (1988)[2,3,4]								
106 White pts			10	23	26	45-37	21-11	
Hochberg (1985)[8,9]								
150 pts								
113 White pts	27		15	32		33	12	≥1.8
37 Black pts	30		24	41		30	11	≥1.95
Jayaram (1990)[4,8]								≥1.36
30 Indian pts	30	73	27	63				
Juby (1991)[2,9]								
108 Canadian pts	22	1	21	36		17	7	
Kiparski (1990)[4]								
(rev in Swaak et al.)			10	20		37	14	
Maddison et al. (1985)[2,3]								
63 UK pts			17-33	30-46		33-55	15-24	
Notman (1975)[1]								
50 pts	70	52	28	26				
Scopelitis et al.(1980)[10,11]								≥1.43
73 Black pts	74		32	40		29	0	
Speransky (1988)[4,10]								≥1.31
107 USSR pts	54		21	27		50	48	
Swaak (1990)[4,10,12]								≥1.03
164 Netherlands	76		9-22	9-29		12-28	18-45	
Westgeest (1990)[4]								
(rev in Swaak et al.)			8-29	8-14		10-16		

Table 10 (continued)

Author	Autoantibody Frequency (%)							Antibody sets/pt
	dsDNA	His	Sm	U1RNP	Sm/U1	Ro	La	
Williams (1990)[4] (rev in Swaak et al.)			19	25		47	5	
Williamson et al.(1983)[6] 71 US pts			32	37		30	11	

[1] ^{3}H
[2]ELISA
[3]immunodiffusion
[4]counterimmunoelectrophoresis
[5]radioimmunoassay and precipitin analysis
[6]immunoprecipitation
[7]western blot
[8]Crithidia
[9]double diffusion
[10]Farr
[11]FANA
[12]Peg

Figure 1

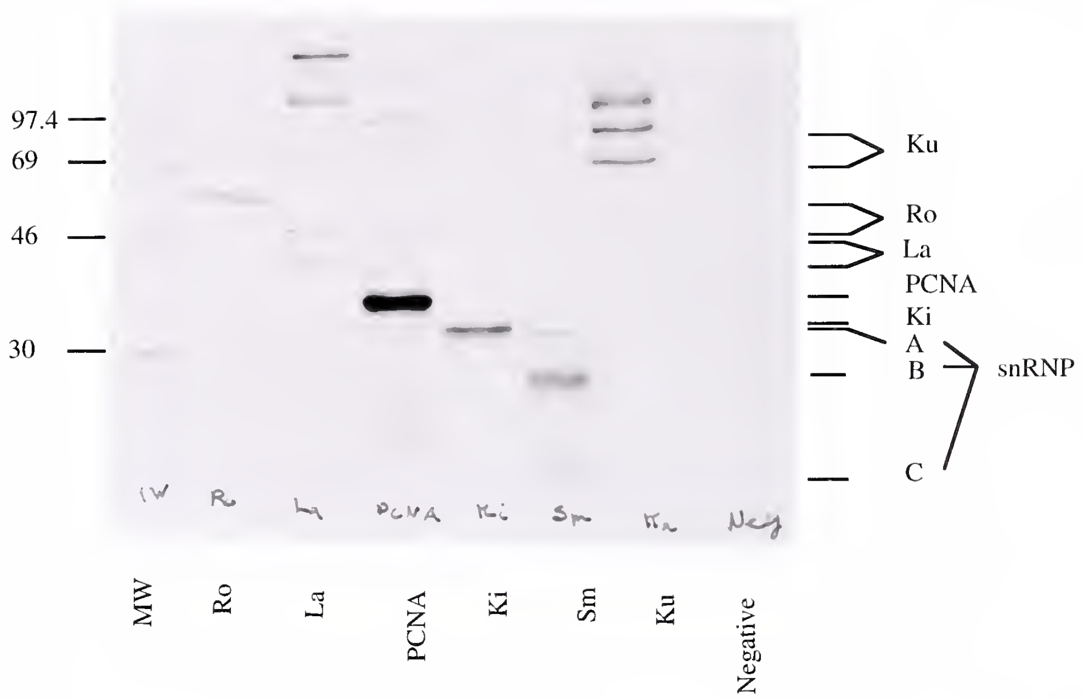

Figure 1. ^{35}S Immunoprecipitation of standard sera of the following specificities by lane: MW marker, Ro, La, PCNA, Ki, Sm, Ku, and normal control.

Figure 2

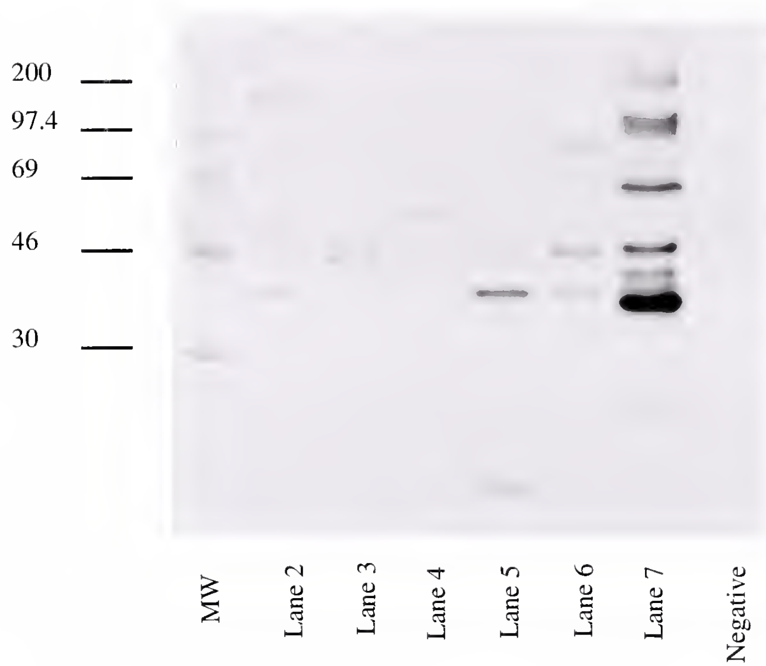

Figure 2. ^{35}S Immunoprecipitation of ribosomal control sera (lanes 2-7) with MW standard in lane 1 and normal control sera in lane 8.

Figure 3

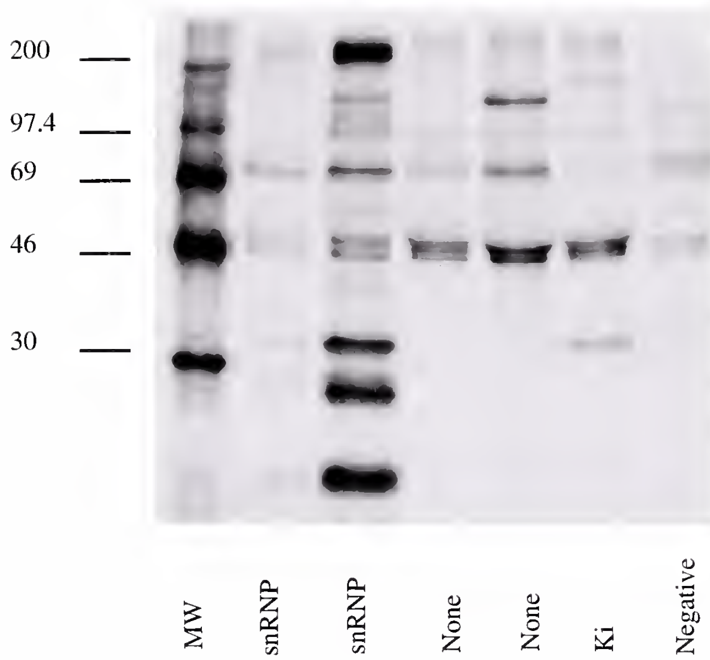

Figure 3. Sample ^{35}S Immunoprecipitation of study population sera showing: MW standard, Sm, Sm, no autoantibodies, no autoantibodies, Ki, and normal control sera.

Figure 4

Distribution of the number of autoantibodies per patient.

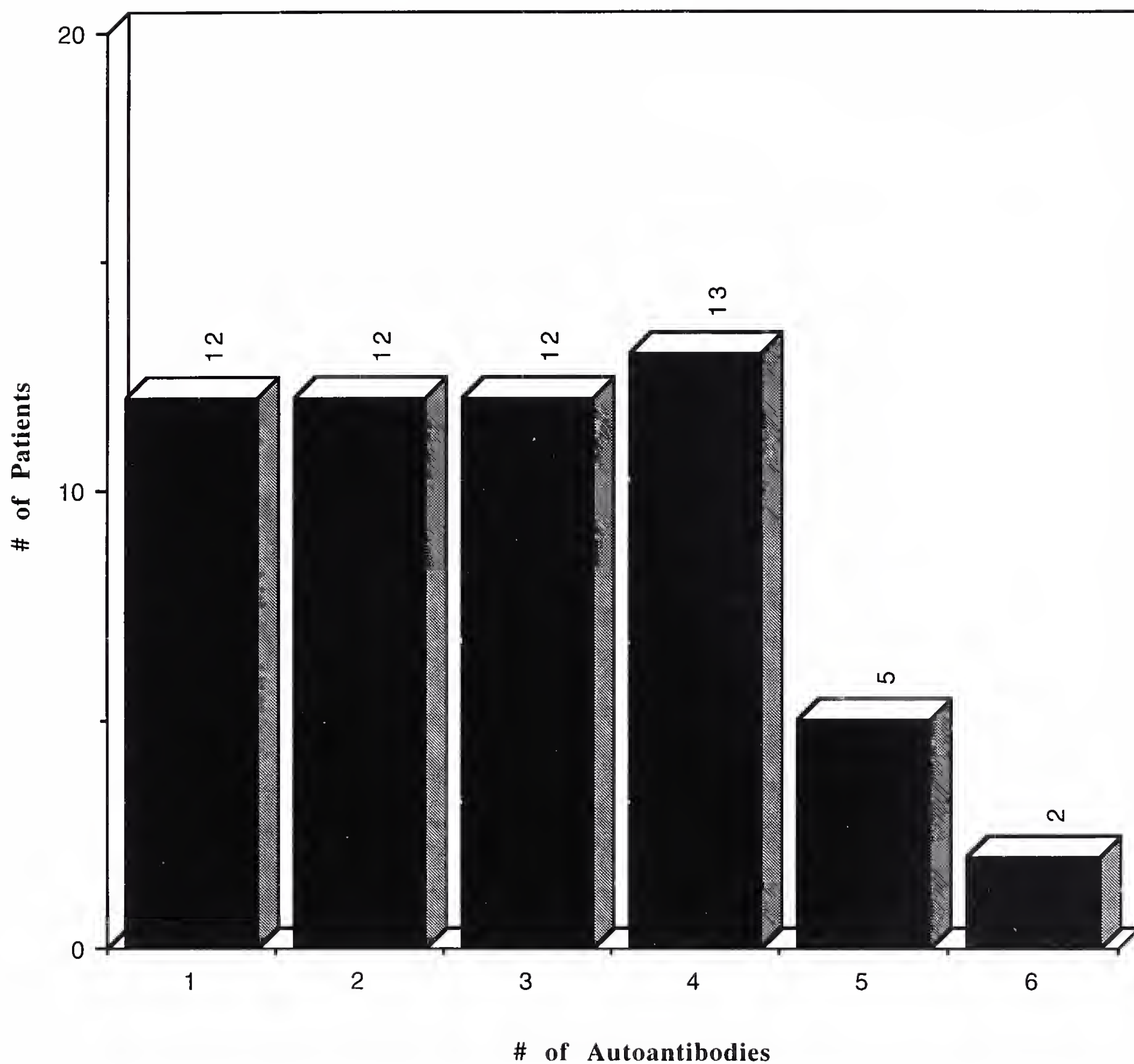

Figure 4. Sera of 68 patients fulfilling the 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus were assayed by ^{35}S immunoprecipitation and ELISA for autoantibodies (dsDNA, histone, Ro, La, Sm, U1 RNP, rRNP, PCNA, Ku, Ki, and/or other) and the number of autoantibodies in each patient serum was calculated. The distribution of the number of patients with each respective number of autoantibodies is shown.

Figure 5

Distribution of the number of autoantibody sets per patient.

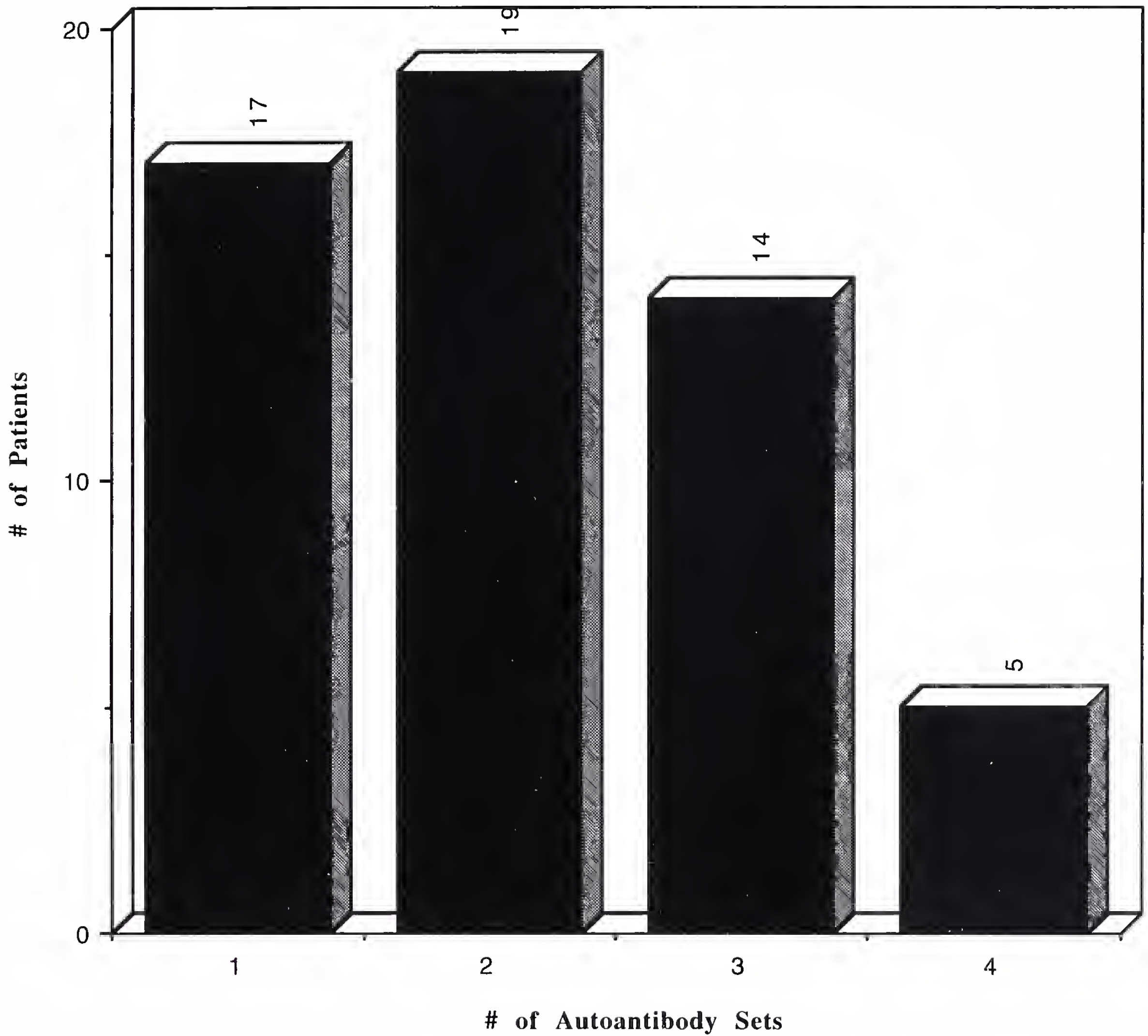

Figure 5. Sera of 68 patients fulfilling the 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus were assayed by ^{35}S immunoprecipitation and ELISA for autoantibody sets (dsDNA/histone, Ro/La, Sm/U1 RNP, rRNP, PCNA, Ku, Ki, and/or other) and the number of autoantibody sets in each patient serum was calculated. The distribution of the number of patients with each respective number of autoantibody sets is shown.

Figure 6

Number of autoantibody sets by duration of disease at phlebotomy.

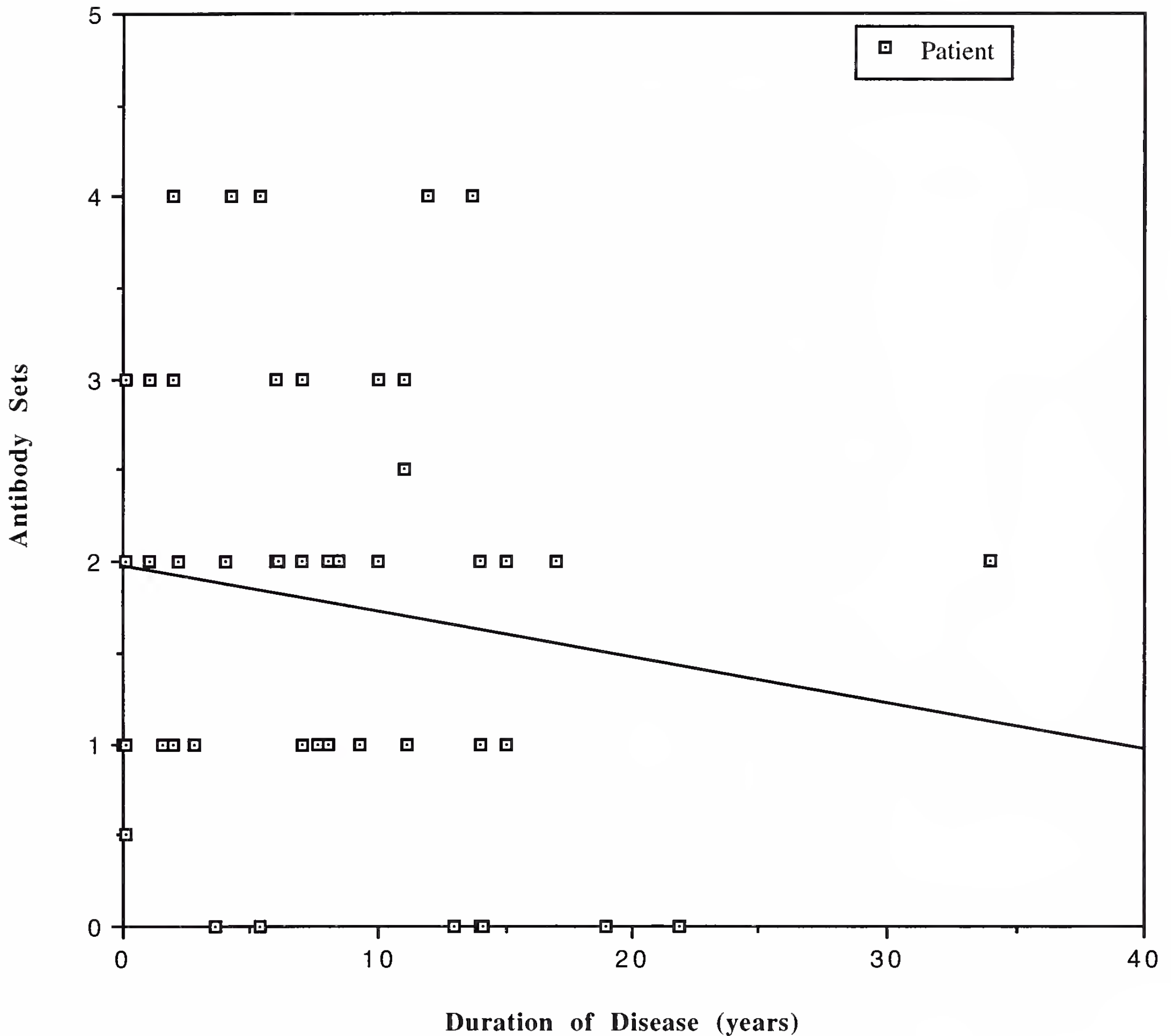

Figure 6. Sera of 68 patients fulfilling the 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus were assayed by ^{35}S immunoprecipitation and ELISA for autoantibody sets (dsDNA/histone, Ro/La, Sm/U1 RNP, rRNP, PCNA, Ku, Ki, and/or other). The duration of disease at time of phlebotomy for each patient was determined by chart review. Each patient is plotted, showing the number of autoantibody sets versus the duration of disease.

DISCUSSION

Using both ^{35}S immunoprecipitation and ELISAs, the autoantibody profiles of 68 patients fulfilling the ARA Revised Criteria for systemic lupus erythematosus were determined. Additionally, autoantibody profiles of SLE patients previously reported in the literature were reviewed and analyzed. The clinical manifestations of disease in the study population were collected through clinical chart review, and Chi-square analysis was used to study possible associations between individual autoantibodies, and between autoantibodies and clinical manifestations.

The prevalence of antibodies to dsDNA (40%), histones (52%), Sm (15%), U1 RNP (34%), Ro (40-43%), and La (15-19%), found using both ^{35}S immunoprecipitation and ELISA, in this previously unreported population are consistent with the literature (Table 1). This confirms that a high uniformity of antibody specificities exists between different populations.

Associations between clinical disease manifestations and autoantibody specificities previously reported in the literature and confirmed in this study include the following: anti-dsDNA antibodies and renal pathology; anti-dsDNA and hypocomplementemia; and anti-U1 antibodies and serositis. Associations previously reported in the literature which were not found in this study include the following: anti-dsDNA antibodies and neurologic disease, malar rash, or hemolytic disease; anti-histone antibodies and photosensitivity; anti-Ro antibodies and rheumatoid factor positivity, vasculitis, or thrombocytopenia; anti-La antibodies and CNS disease, or protection against renal disease; anti-U1 RNP antibodies and vasculitis; and anti-Sm antibodies were not protective against CNS or renal diseases. Interestingly, anti-La antibodies, not anti-Ro antibodies,

correlated with RF positivity which, if the particle hypothesis is true, would support an association between the Ro/La set and RF positivity. Also, anti-histone antibodies, as well as anti-dsDNA antibodies, correlated with hypocomplementemia. Interestingly, anti-Ro antibodies were the only antibodies associated with CNS manifestations of disease.

Additionally, the more prevalent autoantibodies correlated with the more prevalent clinical manifestations, anti-dsDNA antibodies correlated with musculoskeletal disease and serositis, anti-histone antibodies correlated with many clinical manifestations (hemolytic disease, serositis, and musculoskeletal disease), and anti-Ro antibodies correlated with hemolytic disease. Because of the high prevalence of these antibodies and disease manifestations, these associations may be coincidental. Corticosteroid treatment correlated with anti-dsDNA, anti-histone, and anti-Ro antibodies (the three most prevalent antibodies), indirectly suggesting that patients with these particular antibodies, or patients with higher numbers of antibodies, may have more severe disease.

The role of specific autoantibodies in the pathogenesis of the clinical manifestations has long been hypothesized. The present study did not directly address the role of autoantibodies in the pathogenesis of SLE. However, in the present study, anti-dsDNA antibodies correlated with hypocomplementemia and renal pathology which is consistent with a role of dsDNA in the pathogenesis of lupus renal disease. (As stated previously, it is generally believed that anti-dsDNA antibodies form immune complexes which lead to renal injury.) This study did not address a correlation between anti-Ro antibodies and neonatal lupus or subacute cutaneous lupus.

Confirmed in this study are previously reported autoantibody set patterns of the autoimmune response: Ro with La, and dsDNA with histone (all anti-La sera, except one serum, contained anti-Ro, and, of 27 dsDNA positive sera, 22 sera also had anti-histone specificity). Although secondary to the sample size, the study was not able to statistically comment on an association between Sm and U1 RNP, six of ten Sm positive patients had both Sm and U1 RNP. Chi-square analysis most strongly correlated anti-histone and anti-dsDNA antibodies. Anti-histone antibodies also correlated with anti-U1 RNP and anti-Ro antibodies. (Given an average number of three autoantibodies per patient and that these specificities are the most prevalent autoantibody specificities, this association can be explained by statistical probability.) Chi-square analysis also correlated anti-Ro with anti-La antibodies, a commonly found set.

The present study defined more clearly the average number of autoantibody sets per lupus patient than the studies currently in the literature. The current study and 12 identified studies in the literature showed an average of greater than one autoantibody set per patient. Furthermore, it has been shown that severely ill SLE patients can have greater than six autoantibody specificities (Boey et al., 1988). Although this study did not examine disease severity, two patients had six autoantibody specificities. The greatest number of antibody sets found in a single patient was four, which was shown in five patients.

This study supports some theories of etiology over others. At least nine different specificities were demonstrated in this group of SLE patients supporting the generally accepted notion that SLE is manifested by a polyclonal autoantibody response. Secondly, autoantibodies to epitopes on the same macromolecular structure, such as Ro and La, Sm and U1, and

dsDNA and histones, tended to occur in linked sets further supporting that macromolecular structures are targeted in the immune response of SLE. Other research also supports this concept. It has been shown that histone-specific T cells can help anti-dsDNA antibody production. In MRL/lpr mice, antibodies specific for native chromatin are detected before DNA and histone specificities. Craft et al. have shown in normal mice that once T cell tolerance to one snRNP protein is lost, in the presence of spliceosomes, the immune response can expand to target other snRNP proteins. (Craft 1992)

On an average, approximately two sets were found in each patient. These results support a global defect in immune tolerance; therefore, the following etiologic theories appear more likely: (1) polyclonal T cell activation (2) polyclonal B cell activation (3) suppressor T cell defect (4) T_h overactivation (5) role of immunological privileged sites (6) activation of ignorant cells (7) defect in T cell tolerance or (8) defect in B cell tolerance. However, the limited number of autoantibody specificities in the present study suggests that an antigen-directed mechanism is also functioning. This suggests that the etiologic defect is not simply a pure polyclonal T cell activation defect, polyclonal B cell activation defect, defect in T suppressor or T helper cells, or defect in T or B cell tolerance. Other evidence supporting immunogen-directed B cell autoantibody production is outlined by Craft (unpublished), "autoantibodies in lupus are high affinity, high titer, and of IgG isotype. In lupus mice, polyclonal B cell activation precedes specific ANA production." He suggests that autoantibody production may be divided into two steps: polyclonal B cell activation and then clonal selection by self antigens.

The results of this study are not as consistent with the etiologic theories which imply that the autoimmune response is due solely to an antigenically similar cross-reacting antigen, which include the theories of molecular mimicry, cryptic self, and neoself. In order for this data to be consistent with the molecular mimicry model, all antigens targeted by an individuals sera would at some point need to be a part of the same macromolecular structure; more than one molecular mimicry event on average must occur; or the inciting antigen must not be protein specific but charge specific.

The present study has limitations. The study sample size of 68 SLE patients was not adequate to study the prevalence of anti-ribosome P, PCNA, Ku, or Ki antibodies, as well as various clinical manifestations, with statistical significance. This study did not correlate autoantibody patterns, only specific autoantibodies, with clinical manifestations. Furthermore, the utility of studying antibody/clinical manifestations has been questioned (Tan 1989; Craft 1992), and it is important to remember that normal individuals have been shown to possess low levels of antibodies to Ro (15%), La (7.5%), dsDNA, and ssDNA.

The methods used have limitations. Although the prevalence of anti-ribosomal P proteins has been studied with immunoprecipitation in the past (Boey et al., 1988), western blotting is a more specific technique (Elkon et al., 1988). ^{35}S immunoprecipitation labels only those proteins with methionine; therefore, methionine deficient proteins were not adequately detected with the ^{35}S immunoprecipitation method (although U1 RNP, Sm Ro, and La were also detected using ELISAs which are sensitive regardless of methionine content). The utility of Chi-square analysis in the medical literature has been questioned (Jekel, personal communication).

Finally, disease activity and therapy may influence autoantibody levels (Houtman et al., 1985). Forty-three percent of patients in this study were on steroids or anti-nuclear therapy when sera were drawn; active lupus was not a selection criterion for sera used. Therefore, frequencies in this series may not be strictly comparable to other studies in the literature.

The utility of studying autoantibody profiles in SLE patients is multifold. For example, using human autoantibodies, the molecular structure of many autoantigens and cellular processes such as pre-mRNA splicing and DNA replication have been elucidated. Autoantibody profiles have etiologic, diagnostic, and therapeutic utility. The results of this study and studies found in the literature support specific etiologic theories. This study confirmed that several autoantibody specificities can be used to predict particular clinical manifestations. Furthermore, studying autoantibodies has also led to many therapeutic strategies. For example, extended survival in NZW/NZB mice, a murine model of lupus, by the administration of an anti-idiotypic antibody has been demonstrated by Hahn and Ebling (reviewed in Christian and Elkon, 1986).

FURTHER RESEARCH

At least two specific questions arose during the course of this project. First, twenty patients had previously unidentified bands which were considered individual specificities. This may be valid given that it is very possible that these represent previously unidentified specificities. However, it is also possible that these bands represent a degradation product; therefore, leading to a slight overestimation of the number of autoantibody

sets per patient. (The average number of sets, disregarding these bands, is still greater than one.)

Secondly, sixteen patients had no specificities identified. There are several possible explanations: (1) these patients do not have any autoantibodies yet have SLE; (2) these patients do not have either autoantibodies or SLE; (3) the assays used in this study are not adequate to detect the specificities in these sera; or (4) the autoantibodies were degraded while in storage.

These issues represent opportunities of further research and, as newer assays are developed, other investigators may want to re-screen these sera. More globally, this project serves as a reminder that the etiology of SLE is a complex question which has only begun to be answered. Nevertheless, all scientific questions must be answered in parts, and much light-shedding data has been accumulated. This project suggests that further research may best focus on hypotheses that account for a global defect in tolerance.

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