ORIGINAL ARTICLE

Development of Autoantibodies before the Clinical Onset of Systemic Lupus Erythematosus

Melissa R. Arbuckle, M.D., Ph.D., Micah T. McClain, Ph.D., Mark V. Rubertone, M.D., R. Hal Scofield, M.D., Gregory J. Dennis, M.D., Judith A. James, M.D., Ph.D., and John B. Harley, M.D., Ph.D.

ABSTRACT

BACKGROUND

Although much is known about the natural history of systemic lupus erythematosus (SLE), the development of SLE autoantibodies before the diagnosis of the disease has not been extensively explored. We investigated the onset and progression of autoantibody development before the clinical diagnosis.

METHODS

The Department of Defense Serum Repository contains approximately 30 million specimens prospectively collected from more than 5 million U.S. Armed Forces personnel. We evaluated serum samples obtained from 130 persons before they received a diagnosis of SLE, along with samples from matched controls.

RESULTS

In 115 of the 130 patients with SLE (88 percent), at least one SLE autoantibody tested was present before the diagnosis (up to 9.4 years earlier; mean, 3.3 years). Antinuclear antibodies were present in 78 percent (at a dilution of 1:120 or more), anti–double-stranded DNA antibodies in 55 percent, anti-Ro antibodies in 47 percent, anti-La antibodies in 34 percent, anti-Sm antibodies in 32 percent, anti–nuclear ribonucleoprotein antibodies in 26 percent, and antiphospholipid antibodies in 18 percent. Antinuclear, antiphospholipid antibodies, anti-Ro, and anti-La antibodies were present earlier than anti-Sm and anti–nuclear ribonucleoprotein antibodies (a mean of 3.4 years before the diagnosis vs. 1.2 years, P=0.005). Anti–double-stranded DNA antibodies, with a mean onset 2.2 years before the diagnosis, were found later than antinuclear antibodies (P=0.06) and earlier than anti–nuclear ribonucleoprotein antibodies (P=0.005). For many patients, the earliest available serum sample was positive; therefore, these measures of the average time from the first positive antibody test to the diagnosis are underestimates of the time from the development of antibodies to the diagnosis. Of the 130 initial matched controls, 3.8 percent were positive for one or more autoantibodies.

CONCLUSIONS

Autoantibodies are typically present many years before the diagnosis of SLE. Furthermore, the appearance of autoantibodies in patients with SLE tends to follow a predictable course, with a progressive accumulation of specific autoantibodies before the onset of SLE, while patients are still asymptomatic.

From the Arthritis and Immunology Program, Oklahoma Medical Research Foundation (M.R.A., M.T.M., R.H.S., J.A.J., J.B.H.), the Departments of Medicine and Pathology, University of Oklahoma Health Sciences Center (M.T.M., R.H.S., J.A.J., J.B.H.), and the Department of Veterans Affairs (R.H.S., J.B.H.) — all in Oklahoma City; the Department of Rheumatology, Walter Reed Army Medical Center (M.R.A., G.J.D.), and the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health (G.J.D.) — both in Bethesda, Md.; and the U.S. Army Center for Health Promotion and Preventive Medicine, Washington, D.C. (M.V.R.). Address reprint requests to Dr. James at the Arthritis and Immunology Program, Oklahoma Medical Research Foundation, 825 NE 13th St., Oklahoma City, OK 73104, or at jamesj@omrf.ouhsc.edu.

N Engl J Med 2003;349:1526-33.

Systemic lupus erythematosus (SLE) is an autoimmune disease that is virtually always accompanied by the production of autoantibodies. In fact, it has been demonstrated that autoantibodies contribute directly to the pathologic changes of SLE.[1] Since autoantibodies are central to the pathogenesis of the disorder, their development must coincide with or precede clinical disease. Although the prevalence of SLE autoantibodies among patients with confirmed SLE has been established, we know little about the autoimmune history of patients before SLE is diagnosed.

We evaluated a prospectively assembled collection of frozen serum samples to test the hypothesis that the appearance of autoantibodies precedes the diagnosis of SLE. The U.S. Department of Defense Serum Repository contains more than 30 million serum samples. The stringent physical requirements of the U.S. military ensure that subjects are healthy on induction for active duty. A review of military medical records identified 130 persons, some formerly and some currently on active duty, who met the criteria for SLE and for whom stored serum samples obtained before diagnosis were available.

METHODS

SERUM SAMPLES

Since 1985, the Department of Defense Serum Repository has stored serum samples obtained from U.S. Armed Forces personnel on enlistment and, on average, every other year thereafter. The samples are stored at −30°C. Military data bases were searched for records containing the *International Classification of Diseases, 9th Revision, Clinical Modification* (ICD-9-CM) code for SLE (710.0). Records containing this code from 336 persons with serum in the repository were reviewed. Patients were excluded because of inadequate data, insufficient evidence of a diagnosis of SLE,[2] or the absence of prediagnosis serum samples. For each patient with SLE, four controls were randomly selected from among people on active military duty, matched for sex, ethnic group, age (within one year), length of military service, sample availability, and enlistment date (to control for the duration of sample storage).

REVIEW OF MEDICAL RECORDS

Data on clinical and laboratory findings and on sex, ethnic group, date of birth, and date and age at diagnosis were obtained by review of medical records.[2] The presence of each criterion for SLE was determined from the medical records, with many of the diagnostic criteria being documented by military rheumatology referral centers.

The protocol was reviewed and approved by the institutional review board of the Oklahoma Medical Research Foundation and the Human Use Committee of the Walter Reed Army Medical Center. Informed consent for the testing of coded, stored serum samples and the review of records by appropriate military personnel was waived by both institutions. To protect the privacy of the patients, their names and unique personal information were not recorded or released. The dates of the sampling and the analyses ranged from 1992 to 1999.

AUTOANTIBODY ASSAYS

Assays for antinuclear antibodies were performed by indirect immunofluorescence with HEp-2000 cells (Immuno Concepts). Detection of antinuclear antibodies at a dilution of 1:120 was considered a positive result. Enzyme-linked immunosorbent assays were used to evaluate serum for antibodies to Sm, nuclear ribonucleoprotein, Ro, La, and phospholipids (IgG and IgM).[3,4] Values that were 3 SD or more above normal values for background binding were considered positive results. Anti–double-stranded DNA antibodies were screened with a solid-phase assay (Varelisa, Pharmacia Upjohn Diagnostics). All tests yielding equivocal results were repeated, and samples with persistently equivocal results for anti–double-stranded DNA were tested with a crithidia immunofluorescence assay (Protrac Industries).[5]

STATISTICAL ANALYSIS

Categorical variables (such as ethnic group and sex) were assessed by the chi-square statistic. For each type of autoantibody, the time from autoantibody positivity to the diagnosis of SLE was calculated on the basis of the date of the first positive antibody test and the date of diagnosis. Patients in whom antibodies developed before diagnosis were assigned negative values for the time between antibody development and diagnosis, and patients in whom antibodies developed after diagnosis were assigned positive values for the time between antibody development and diagnosis. For patients whose first available serum sample yielded a positive antibody-test result, this time represents a lower boundary for the duration of positivity before the diagnosis of SLE.

For each antibody, the mean time from the first recorded positive test to the diagnosis of SLE was

calculated on the basis of data from all patients in whom that antibody had developed at any time. Student's t-test was used to test for differences between antibodies with respect to the mean time from the first positive test to the diagnosis. The antibodies were sorted into three groups: early-, intermediate-, and late-appearing antibodies. Within each group, the mean time from the development of antibodies to the diagnosis of SLE did not differ significantly among antibodies ($P>0.05$). The mean time from antibody development to diagnosis for each of the three groups of antibodies was determined by computing averages weighted according to the number of persons with each antibody.

The time between antibody development and the appearance of the first American College of Rheumatology clinical criterion for SLE was also calculated and analyzed. The mean and median values for the time from the appearance of the first clinical criterion to the diagnosis of SLE were not similar to each other, unlike the other values analyzed. Means are therefore presented for all values, except for the time from the appearance of the first clinical criterion to the diagnosis of SLE, for which mean and median values are presented.

Kaplan–Meier product–limit survival curves[6,7] were constructed for the time from the initial positive serum sample to the time of diagnosis and to the appearance of the first American College of Rheumatology clinical criterion. Data from patients with a positive autoantibody test for the earliest serum sample available for testing were treated as censored observations at the time of the first serum sample. The differences between autoantibodies in the time from the first positive test to the appearance of the first clinical criterion for SLE were then evaluated with the use of Gehan's generalized Wilcoxon test.[7-9]

RESULTS

PATIENT POPULATION

A diagnosis of SLE was established in 130 military personnel, some formerly and some currently on active duty, for whom serum samples obtained before diagnosis were available. Of these 130 patients, 36 percent were men, 62 percent were black, 26 percent were of European ethnic background, 10 percent were Hispanic, and 2 percent were Asian. The mean (±SD) age at diagnosis was 30.4±6.8 years (range, 18.5 to 46.9). A mean of 4.9±2.5 serum samples were available for each patient (range, 1 to 12). The earliest available serum sample for each patient was obtained a mean of 4.4±2.5 years before the diagnosis (with a maximal interval of 9.4 years). Serum samples obtained after the diagnosis (up to six years afterward) were also available from 77 patients (59 percent). For the analysis of autoantibodies detected after the diagnosis, the data were censored to reflect the loss to follow-up of persons after the time of collection of the last available serum sample.

AUTOANTIBODY PREVALENCE

A total of 633 serum samples from patients and 390 samples from controls were evaluated for autoantibody binding with the use of assays for anti-

Table 1. Detection of Autoantibodies before Diagnosis and before the Onset of Symptoms in 130 Patients with Systemic Lupus Erythematosus.*

Autoantibody	Positive Test before Diagnosis	Time from First Detection to Diagnosis	Positive Test in First Serum Sample	Total Patients with Positive Test	Interval between Positive Test and Diagnosis	Positive Test before Onset of Symptoms†	Interval between Positive Test and Onset of Symptoms
	no. (%)	*yr*	*%*	*no. (%)*	*yr*	*no. (%)*	*yr*
Antinuclear antibodies	101 (78)	9.2	50	109 (84)	3.01±0.25	89 (77)	2.25±0.27
Anti-Ro antibodies	61 (47)	9.4	64	64 (49)	3.68±0.34	55 (48)	2.97±0.39
Anti-La antibodies	44 (34)	8.1	62	45 (35)	3.61±0.38	39 (34)	2.83±0.43
Antiphospholipid antibodies	24 (18)	7.6	67	27 (21)	2.94±0.50	19 (17)	2.29±0.56
Anti–double-stranded DNA antibodies	72 (55)	9.3	48	80 (62)	2.24±0.31	54 (47)	1.24±0.31
Anti-Sm antibodies	41 (32)	8.1	31	49 (38)	1.47±0.34	28 (24)	0.47±0.44
Anti–nuclear ribonucleoprotein antibodies	34 (26)	7.2	23	43 (33)	0.88±0.32	23 (20)	0.20±0.47

* Plus–minus values are means ±SE.
† The percentages are based on data from the 115 patients who had serum samples available from before the onset of symptoms.

nuclear antibodies or specific antigens. Antinuclear antibodies were the most prevalent autoantibodies in serum samples obtained before the diagnosis, occurring in 78 percent of the patients, but other autoantibodies were also frequently found before the diagnosis (Table 1). In fact, most of the patients with a specific type of autoantibody had a positive test for that autoantibody before the diagnosis (Table 1).

Of the 130 initial matched controls, 3.8 percent were positive for one or more autoantibodies (3 percent for anti–double-stranded DNA, 3 percent for anti-Ro, 2 percent for antiphospholipid, and 2 percent for anti–nuclear ribonucleoprotein antibodies). These results are similar to those previously published.[10-13] Antinuclear antibodies were detected in 9.2 percent of samples from the matched controls at a dilution of 1:40, but in none of the control samples at a dilution of 1:120, the criterion used in this study. No control samples were positive for anti-Sm or anti-La antibodies.

Samples from an additional 130 matched controls were also tested for antibodies at two separate times, a mean of 4.69 years apart. None of these control samples had antinuclear antibodies (at a 1:120 dilution), anti-Sm antibodies, or anti-La antibodies. One control had anti-Ro antibodies. Anti–nuclear ribonucleoprotein or antiphospholipid antibodies developed in two other controls, and two controls with initially positive tests subsequently had negative tests.

TIME FROM THE DEVELOPMENT OF AUTOANTIBODIES TO THE DIAGNOSIS OF SLE

For each subject, the first positive test was identified for each autoantibody. In a substantial number of patients with SLE (90 of 130, or 69 percent), antibodies were detected in the first available serum sample, and therefore the time from the development of autoantibodies to the diagnosis of SLE was longer than our estimate. At least one SLE autoantibody was present before diagnosis (up to 9.4 years earlier; mean, 3.3) in 115 patients (88 percent). The mean interval between the earliest autoantibody detection and diagnosis ranged from 3.68 years for anti-Ro antibodies to 0.88 year for anti–nuclear ribonucleoprotein antibodies (Table 1). The antibodies could be clearly separated into three groups according to the length of this interval. The intervals between the first detection of antibodies and the diagnosis of SLE for antinuclear, antiphospholipid, anti-Ro, and anti-La antibodies were statistically indistinguishable from one another (Table 2) and were

Table 2. Relative Timing of the Development of Autoantibodies in Patients with Systemic Lupus Erythematosus.*

Antibody	Antinuclear Antibodies	Anti-Ro Antibodies	Anti-La Antibodies	Antiphospholipid Antibodies	Anti–Double-Stranded DNA Antibodies	Anti-Sm Antibodies	Anti–Nuclear Ribonucleoprotein Antibodies
	years						
Antinuclear	—						
Anti-Ro	0.65	—					
Anti-La	0.60	–0.07	—				
Antiphospholipid	–0.04	–0.71	–0.64	—			
Anti–double-stranded DNA	–0.77	–1.44†	–1.37‡	–0.73	—		
Anti-Sm	–1.54§	–2.21§	–2.14§	–1.50¶	–0.77	—	
Anti–nuclear ribonucleoprotein	–2.13§	–2.80§	–2.73§	–2.09§	–1.36‖	–0.59	—

* The numbers represent the mean time (in years) between the appearance of the antibodies listed in the left-hand column and those listed at the top of the other columns. Positive numbers indicate that the antibody listed on the left appeared before the antibody listed on the top, and negative numbers indicate that the antibody listed on the top appeared first. P values were calculated with the use of two-tailed Student's t-tests. P values are for individual tests and are not appropriate for joint consideration.
† P=0.002.
‡ P=0.006.
§ P<0.001.
¶ P=0.02.
‖ P=0.005.

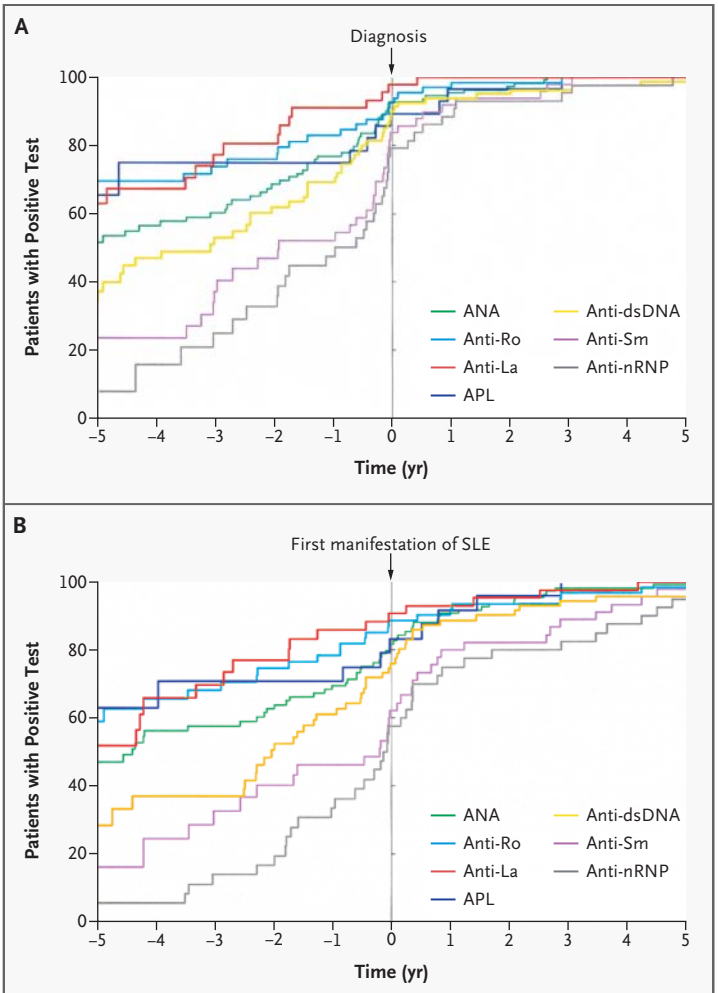

Figure 1. Kaplan–Meier Product–Limit Curves for the Proportion of Patients with Positive Antibody Tests Relative to the Time of Diagnosis or Appearance of the First Clinical Manifestation of Systemic Lupus Erythematosus (SLE).

For each autoantibody, the proportion of patients testing positive relative to the time of diagnosis or to the time of appearance of the first clinical criterion was assessed. In the analyses of the time from antibody development to the diagnosis of SLE (Panel A), antinuclear antibodies (ANA) appeared significantly earlier than anti-Sm antibodies ($Z=3.22$, $P<0.001$) and anti–nuclear ribonucleoprotein antibodies (anti-nRNP) ($Z=4.18$, $P<0.001$) but not significantly earlier than anti-Ro, anti-La, antiphospholipid (APL), or anti–double-stranded DNA antibodies (anti-dsDNA). In the analyses of the time from antibody development to the first clinical manifestation (Panel B), antinuclear antibodies appeared significantly earlier than anti-Sm antibodies ($Z=2.98$, $P=0.003$) and anti–nuclear ribonucleoprotein antibodies ($Z=4.34$, $P<0.001$) but not significantly earlier than the other autoantibodies, with anti–double-stranded DNA antibodies being intermediate ($P=0.06$).

longer than those for anti-Sm and anti–nuclear ribonucleoprotein antibodies (mean, 3.4 vs. 1.2 years; $P=0.005$). Anti–double-stranded DNA antibodies were first detected a mean of 2.2 years before diagnosis, which was later than the first detection of antinuclear antibodies ($P=0.06$) and earlier than that of anti–nuclear ribonucleoprotein antibodies ($P=0.005$).

In a substantial proportion of cases, autoantibodies were present in the earliest available serum sample and were therefore never documented as having been undetectable (Table 1). To allow for patients with positive results in the first sample who may actually have had autoantibodies much earlier, we used Kaplan–Meier product–limit curves (Fig. 1) to evaluate the change in the proportion of patients with positive results over time.

The proportion of patients with SLE who had anti-Sm or anti–nuclear ribonucleoprotein antibodies increased dramatically in the year before the diagnosis. Among patients who ever had a positive autoantibody result, the rate of seroconversion was approximately 20 percent during the year before diagnosis for antinuclear, anti-Ro, or anti-La antibodies and 30 percent for anti–double-stranded DNA antibodies. In contrast, the rate of initial detection in the year before the diagnosis was 82 percent for anti-Sm antibodies and 75 percent for anti–nuclear ribonucleoprotein antibodies. These findings reflect the close temporal relation between the development of these autoantibodies and clinical disease.

TIME FROM THE DEVELOPMENT OF AUTOANTIBODIES TO THE APPEARANCE OF THE FIRST CLINICAL CRITERION

In 27 patients, the first documented appearance of one of the clinical criteria of SLE occurred in the same month as the diagnosis of SLE. Most patients had a more insidious onset of disease; 21 of 130 (16 percent) presented with a clinical symptom more than three years before the diagnosis, and a few presented with a clinical symptom as much as a decade before the diagnosis. These data are somewhat skewed, and, on average, the patients presented with the first clinical symptom 1.5 years before the diagnosis (median, 0.42 year).

Since nearly all patients also acquired autoantibodies before the diagnosis of SLE, we calculated the time from the appearance of individual autoantibodies to the appearance of any clinical manifestation of SLE. Serum samples obtained before the appearance of any clinical manifestation of SLE were available for 115 of the 130 patients. In most of the antibody-positive patients (90 percent), antibodies developed before the appearance of the first clinical manifestation. Indeed, analysis of the data accord-

ing to the time from the first detection of each antibody to the onset of the first clinical (nonantibody) criterion for SLE showed the progressive nature of this disorder (Table 1 and Fig. 1 and 2). Over 90 percent of patients who were ever positive for antinuclear, anti-Ro, anti-La, antiphospholipid, or anti–double-stranded DNA antibodies had a positive test long before the first clinical manifestation of SLE. However, the initial detection of anti–nuclear ribonucleoprotein and anti-Sm antibodies (mean interval before diagnosis, 1.2 years) tended to coincide with the onset of clinical manifestations of SLE (mean interval, 1.5 years).

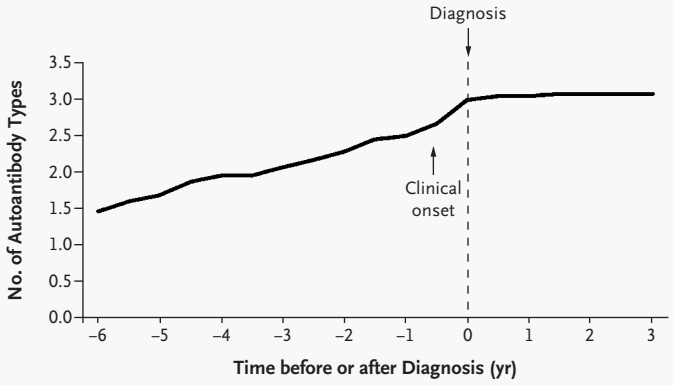

Figure 2. Accumulation of Systemic Lupus Erythematosus Autoantibodies.
The curve shows the average number of types of autoantibody in relation to the time of diagnosis of systemic lupus erythematosus. Seven autoantibodies were evaluated, which bind cellular constituents (antinuclear antibodies), Ro, La, double-stranded DNA, Sm, phospholipid, and nuclear ribonucleoprotein. The time of diagnosis and the median time of the first appearance of any clinical criterion useful for the classification of systemic lupus erythematosus (clinical onset) are indicated by arrows.

ACCRUAL OF AUTOANTIBODIES

The rate of appearance of new types of autoantibodies gradually increased up to the diagnosis of SLE. This accrual of antibodies in the year before diagnosis virtually stopped at diagnosis (Fig. 2). Six years before the diagnosis, patients had, on average, 1.47 of the 7 types of antibodies measured in this study. This number increased to 2.58 with the appearance of the first clinical criterion and then to 3.01 at diagnosis. This process of accrual of autoantibody specificities halted at diagnosis, with only 3.07 specificities present as late as five years after diagnosis.

DISCUSSION

The prospectively assembled Department of Defense Serum Repository is a large, unique resource that has provided an opportunity to examine the development of autoimmunity before the onset of clinical illness in patients with SLE. A number of important lessons are clear from these observations. Some autoantibodies (antinuclear, anti-Ro, anti-La, and antiphospholipid antibodies) usually precede the onset of SLE by many years. Others (anti-Sm and anti–nuclear ribonucleoprotein antibodies) typically appear only months before diagnosis, during the time when characteristic clinical manifestations appear. Anti–double-stranded DNA antibodies are intermediate between these two groups of antibodies. This pattern is consistent with the known reports of positive tests for antinuclear, anti-Ro, anti-La, and antiphospholipid antibodies before the diagnosis of SLE,[14-19] and with the virtual absence of reports of positive tests for anti-Sm and anti–double-stranded DNA antibodies before the clinical diagnosis.

Our findings also correlate with the observed frequency of these autoantibodies in the normal population and their known association with disease activity.[20,21] Anti-Ro, anti-La, antiphospholipid, and antinuclear antibodies are in fact relatively common in normal persons who never have clinical symptoms of rheumatic disease. In contrast, anti–double-stranded DNA, anti-Sm, and anti–nuclear ribonucleoprotein antibodies are very rare in normal persons.[13,22] We found that the interval between the first positive test for each of these three autoantibodies and the initial clinical manifestation of disease was shorter than that for anti-Ro, anti-La, antiphospholipid, and antinuclear antibodies.

A half-century ago, when the diagnosis was first made with confidence, the five-year mortality among patients with SLE was 50 percent.[23] Dubois argued that corticotropin and corticosteroids were responsible for the dramatic improvement in survival between the 1950s and the 1970s.[24] Our results show that new autoantibodies steadily accumulate before the diagnosis and cease to accumulate thereafter (Fig. 2), perhaps as a result of unknown aspects of the natural history of the disease or of the modern treatments typically used when SLE is diagnosed.

SLE tends to arise in asymptomatic persons with positive serologic tests for SLE-associated autoantibodies. The extent to which the risk among those with positive serologic tests exceeds the overall rate of 5.6 per 100,000 per year[25] can be only crudely es-

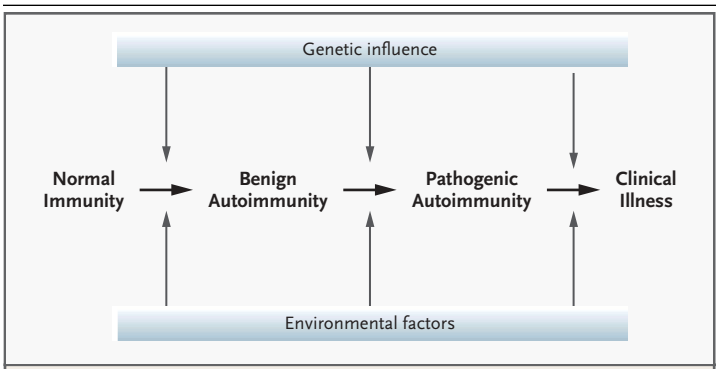

Figure 3. Phases in the Development of Pathogenic Autoimmunity.
Normal immunity progresses to benign autoimmunity through the influence of genetic composition and environment. Later, benign autoimmunity progresses to pathogenic autoimmunity. Symptoms of clinical illness appear soon after pathogenic autoimmunity develops.

timated from our data. The presence of antinuclear antibodies (at a dilution of 1:120 or more) or anti-Ro, anti-La, or antiphospholipid antibodies appears to increase the risk by a factor of at least 40 (according to their relative frequency in the normal controls in our study); however, their presence does not suggest that the onset of clinical illness is imminent. Our findings do not address the question of whether asymptomatic persons incidentally discovered to have SLE-specific autoantibodies (anti-Sm or anti–double-stranded DNA antibodies) should be monitored or treated.

Our results are consistent with data from prospective studies of asymptomatic women with positive tests for anti-Ro antibodies who gave birth to babies with neonatal SLE manifested as complete congenital heart block. After 5 to 10 years of follow-up, either Sjögren's syndrome or SLE developed in many of these women.[26] In a 10-year follow-up of healthy subjects with a positive test for rheumatoid factor, rheumatoid arthritis developed in only 9 of 129, but this rate was 40 times as high as that among subjects without rheumatoid factor.[27] There are also prospective data from persons with positive tests for diabetes-associated autoantibodies showing that 70 to 80 percent of those in whom type 1 diabetes later developed could be identified by using a panel of autoantibodies.[28] In all of these diseases, as in SLE, there is a tendency for the appearance of autoantibodies to precede the clinical onset of disease, a finding that underscores the potential importance of autoantibodies in the pathogenesis of these diseases.

Even though our study suggests that immune events occur years before the diagnosis of SLE, our findings should be interpreted in the context of the limitations of the data. The estimates of time provided by this study are biased by the substantial proportion of patients (69 percent) whose data were censored because autoantibodies were present in the first available serum sample (obtained a mean of four years before the diagnosis). If serum samples obtained before the development of autoantibodies had been available for all patients, our estimates of the mean time from autoantibody development to the diagnosis would have been longer. Consequently, this study provides a lower-boundary estimate of the time before the diagnosis at which particular autoantibodies develop.

Our serologic and clinical findings, along with those of previous studies,[29] suggest that there are at least three phases in the development of SLE autoantibodies (Fig. 3). In the first, or normal, phase, are asymptomatic persons with no SLE autoantibodies. Only 32 of the 130 patients in whom SLE developed (25 percent) were found to be in this phase at the time of the first serum sample. In the second phase, benign autoimmunity, there is a laboratory finding but without immediate clinical manifestations. Antinuclear, anti-Ro, anti-La, or antiphospholipid antibodies are most likely to be present during this phase. The third phase, pathogenic autoimmunity, is marked by the presence of the more ominous autoantibodies — namely, anti–double-stranded DNA, anti-Sm, and anti–nuclear ribonucleoprotein antibodies — and by the onset of signs and symptoms leading to clinical presentation and diagnosis.

This concept of a crescendo of autoimmunity culminating in clinical illness is also supported by data showing increased concentrations of autoantibodies before diagnosis[30] and progressive accrual of autoantibody specificities at the epitope level. The anti-Sm response, for example, appears to be elicited first by a single antigenic structure. The responses to the first few additional epitopes follow a specific sequence of immune structural recognition. These responses eventually mature (over a period of approximately two years) into a more idiosyncratically complex reaction that binds an average of eight epitopes of Sm B.[31,32]

Our results show that clinical SLE is preceded by complicated autoimmune changes that are usually under way for many years before diagnosis. Antinuclear, anti-Ro, anti-La, and antiphospholipid anti-

bodies appear first, followed by anti–double-stranded DNA antibodies, and then by anti-Sm and anti–nuclear ribonucleoprotein antibodies. The number of autoantibody types continues to increase until the time of diagnosis and therapeutic intervention. SLE, then, is the culmination of compound autoimmune abnormalities that begin simply, perhaps even as isolated immunologic events, and that spread and multiply until they are manifested as a potentially devastating clinical disease.

Supported by grants from the National Institutes of Health (AI31584, RR15577, AR4904, AR48940, AR01981, AR45084, AR45231, AR42460, AI24717, and RR14467 [for biostatistical support]) and the Department of Veterans Affairs.

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Departments of the Army, Navy, or Defense.

We are indebted to Ben Bruner, Tara Bruner, Roy Rindler, and Xana Kim for their technical assistance; to Drs. Christie Burgin, Don Parker, and Chris Aston for statistical assistance; and to Kimmie Kohlhase and the cooperating military rheumatologists and rheumatology clinics for case identification.

REFERENCES

1. Reichlin M, Harley JB. Antibodies to Ro/SSA and La/SSB. In: Wallace DJ, Hahn BH, eds. Dubois' lupus erythematosus. 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2002:467-80.

2. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.

3. Gharavi AE, Harris EN, Asherson RA, Hughes GR. Anticardiolipin antibodies: isotype distribution and phospholipid specificity. Ann Rheum Dis 1987;46:1-6.

4. McClain MT, Ramsland PA, Kaufman KM, James JA. Anti-Sm autoantibodies in systemic lupus target highly basic surface structures of complexed spliceosomal autoantigens. J Immunol 2002;168:2054-62.

5. Aarden A, de Groot ER, Feltkamp TEW. Immunology of DNA. III. *Crithidia luciliae*, a simple substrate for the determination of anti-dsDNA with the immunofluorescence technique. Ann N Y Acad Sci 1975;254:505-15.

6. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.

7. Lee ET. Statistical methods for survival data analysis. 2nd ed. New York: John Wiley, 1992.

8. Gehan EA. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 1965;52:203-23.

9. *Idem*. A generalized two-sample Wilcoxon test for doubly censored data. Biometrika 1965;52:650-3.

10. Isenberg DA, Dudeney C, Williams W, et al. Measurement of anti-DNA antibodies: a reappraisal using five different methods. Ann Rheum Dis 1987;6:448-56.

11. Avina-Zubieta JA, Galindo-Rodriguez G, Kwan-Yeung L, Davis P, Russell AS. Clinical evaluation of various selected ELISA kits for the detection of anti-DNA antibodies. Lupus 1995;4:370-4.

12. Azizah MR, Azila MN, Zulkifli MN, Norita TY. The prevalence of antinuclear anti-dsDNA, anti-Sm and anti-RNP antibodies in a group of healthy blood donors. Asian Pac J Allergy Immunol 1996;14:125-8.

13. Gaither KK, Fox OF, Yamagata H, Mamula MJ, Reichlin M, Harley JB. Implications of anti-Ro/Sjogren's syndrome A antigen autoantibody in normal sera for autoimmunity. J Clin Invest 1987;79:841-6.

14. Vlachoyiannopoulos PG, Tzavara V, Dafni U, Spanos E, Moutsopoulos HM. Clinical features and evolution of antinuclear antibody positive individuals in a rheumatology outpatient clinic. J Rheumatol 1998;25:886-91.

15. Mosca M, Tavoni A, Neri R, Bencivelli W, Bombardieri S. Undifferentiated connective tissue diseases: the clinical and serological profiles of 91 patients followed for at least 1 year. Lupus 1998;7:95-100.

16. Press J, Uziel Y, Laxer R, Luy L, Hamilton RM, Silverman ED. Long-term outcome of mothers of children with complete congenital heart block. Am J Med 1996;100:328-32.

17. Julkunen H, Kurki P, Kaaja R, et al. Isolated congenital heart block: long-term outcome of mothers and characterization of the immune response to SS-A/Ro and SS-B/La. Arthritis Rheum 1993;36:1588-98.

18. Simmons-O'Brien E, Chen S, Watson R, et al. One hundred anti-Ro (SS-A) antibody positive patients: a 10-year follow-up. Medicine (Baltimore) 1995;74:109-30.

19. Satoh M, Yamagata H, Watanabe F, et al. Development of anti-Sm and anti-DNA antibodies followed by clinical manifestation of systemic lupus erythematosus in an elderly woman with long-standing Sjogren's syndrome. Lupus 1995;4:63-5.

20. Spronk PE, Limburg PC, Kallenberg CGM. Serologic markers of disease activity in systemic lupus erythematosus. Lupus 1995;4:86-94.

21. Elkon KB. Autoantibodies in SLE. In: Klippel JH, Dieppe PA, eds. Rheumatology. 2nd ed. Vol. 2. Section 7. London: Mosby, 1998:5.1–5.10.

22. Zarmbinksi MA, Messner RP, Mandel JS. Anti-dsDNA antibodies in laboratory workers handling blood from patients with systemic lupus erythematosus. J Rheumatol 1992;19:1380-4.

23. Harvey AM, Schulman LE, Tumulty PA, Conley CL, Schoenrich EH. Systemic lupus erythematosus: review of the literature and clinical analysis of 38 cases. Medicine (Baltimore) 1954;33:291-437.

24. Dubois EL. Results of steroid therapy in systemic lupus erythematosus. In: Dubois EL, ed. Lupus erythematosus: a review of the current status of discoid and systemic lupus erythematosus and their variants. 2nd ed. Los Angeles: University of Southern California Press, 1974:613-32.

25. Ruiz-Irastorza G, Khamashta MA, Castellino G, Hughes GRV. Systemic lupus erythematosus. Lancet 2001;357:1027-32.

26. Julkunen H, Eronen M. Long-term outcome of mothers of children with isolated heart block in Finland. Arthritis Rheum 2001;44:647-52.

27. Aho K, Heliövaara M, Maatela J, Tuomi T, Palosuo T. Rheumatoid factors antedating clinical rheumatoid arthritis. J Rheumatol 1991;18:1282-4.

28. Batstra MR, Aanstoot HJ, Herbrink P. Prediction and diagnosis of type 1 diabetes using beta-cell autoantibodies. Clin Lab 2001;47:497-507.

29. Aho K, Koskela P, Makitalo R, Heliovaara M, Palosuo T. Antinuclear antibodies heralding the onset of systemic lupus erythematosus. J Rheumatol 1992;19:1377-9.

30. Arbuckle MR, James JA, Kohlhase KF, Rubertone MV, Dennis GJ, Harley JB. Development of anti-dsDNA autoantibodies prior to clinical diagnosis of systemic lupus erythematosus. Scand J Immunol 2001;54:211-9.

31. James JA, Gross T, Scofield RH, Harley JB. Immunoglobulin epitope spreading and autoimmune disease after peptide immunization: Sm B/B′-derived PPPGMRPP and PPPGIRGP induce spliceosome autoimmunity. J Exp Med 1995;181:453-61.

32. Arbuckle MR, Reichlin M, Harley JB, James JA. Shared early autoantibody recognition events in the development of anti-Sm B/B′ in human lupus. Scand J Immunol 1999;50:447-55.