Anti-Nucleic Acid Antibodies in Systemic Lupus Erythematosus Patients and Their Families

INCIDENCE AND CORRELATION WITH LYMPHOCYTOTOXIC ANTIBODIES

RAPHAEL J. DEHORATIUS, RAO PILLARISETTY, RONALD P. MESSNER, and NORMAN TALAL

From the Divisions of Rheumatology, Departments of Medicine, Veterans Administration Hospitals, Albuquerque, New Mexico 87108 and San Francisco, California 94121, and the Departments of Medicine, Universities of New Mexico, Albuquerque, New Mexico 87131, and California, San Francisco, California 94122

ABSTRACT Anti-RNA antibodies were found in 82% of 28 systemic lupus erythematosus (SLE) probands and in 16% of 124 of their family members. The incidence in 76 control family members was only 5%. In the SLE family members, the antibodies were found exclusively in 21% of the 94 close household contacts of the probands. The incidence of anti-native DNA (nDNA) antibodies was 68% for the SLE probands. The incidence of anti-nDNA antibodies in close household contacts of the probands was 6%, which was not significantly different from the 1% incidence found in control families. Lymphocytotoxic antibodies occurred in 57% of the SLE family members as a whole and in 68% of the close household contacts. In the SLE probands, lymphocytotoxic antibodies correlated with anti-single-stranded RNA (poly A) and anti-nDNA but not with anti-double-stranded RNA (poly A·poly U). On the other hand, lymphocytotoxic antibodies in the household contacts correlated with anti-double-stranded RNA (poly A·poly U) but not with anti-poly A or anti-nDNA. The anti-RNA antibodies were present in consanguineous household contacts but not in nonconsanguineous household contacts. These findings strengthen the hypothesis that both an environmental agent, possibly a virus, as well as the genetic response are important in the pathogenesis of SLE. Family members may therefore be a logical population in whom to search for specific antibodies to a viral agent.

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INTRODUCTION

Immunologic and viral factors are important in the etiology and pathogenesis of systemic lupus erythematosus (SLE)[1] (1–3). Studies in the New Zealand black (NZB) mouse (4) as well as in the dog model for SLE (5) have implicated viral factors in the etiology and pathogenesis of the disease that develops in these animals. Lymphocytotoxic antibody is found both in the NZB mouse (6) and in a large percentage of SLE patients (7). It also occurs in a significant proportion of family members of SLE patients, especially in individuals with close household contact (8). SLE patients have elevated antibody titers to a variety of viruses (9, 10), to reovirus RNA (11), and to single- and double-stranded RNA (12). Similar anti-nucleic acid antibodies, both anti-RNA and anti-DNA, are found in the NZB mouse model for SLE (13). The present study had two purposes: to determine if anti-nucleic acid antibodies occur in lupus family members, and if so, whether any correlation exists between specific anti-nucleic acid antibodies and lymphocytotoxic antibodies either in the SLE patients or in their relatives.

METHODS

A total of 26 families, containing 28 patients with SLE, were studied. The composition of this group has been described previously (8). Sera were obtained from 124 family

[1] *Abbreviations used in this paper:* nDNA, native DNA; NZB, New Zealand black; poly A, polyadenylic acid; poly U, polyuridylic acid; SLE, systemic lupus erythematosus.

members. 94 family members had close household contact with the SLE proband while 30 did not have close household contact. Household contacts were defined as having close daily contact with the proband before the onset and/or during the course of their disease (8). 20 of the household contacts were related to the proband only by marriage. Nonhousehold contacts had only casual contact with the proband or lived in distant cities.

86 relatives were first-degree relatives, defined as parents, children, and siblings. These first-degree relatives comprised 81% of the total 106 first-degree relatives of the probands over 7 yr of age. 68 of these 86 first-degree relatives had close household contact. 18 were second-degree relatives, defined as twice removed from the proband (i.e. grandparents, uncles, aunts, nieces, and nephews). 20 SLE family members were related only by marriage (nonconsanguineous), and all 20 had close household contact with the probands. Control sera were obtained from 76 individuals comprising 94% of the members of 17 families. These families and the SLE families were matched with respect to age, sex, parity, ethnic background, socioeconomic status, and geographic area (8). Neither the control individuals nor the SLE family members had signs or symptoms of SLE. None of the subjects had an acute viral illness at the time of study.

Nucleic acid antibodies were determined as previously described (14). Control and SLE family sera were analyzed for nucleic acid antibodies simultaneously, with the relationship of the subjects unknown to the investigator performing these assays. Before antibody assay, 10 μl of serum was diluted with buffer to a final volume of 90 μl and incubated at 56°C for 30 min to destroy complement components which can bind nucleic acids nonspecifically. 10 μl of the radiolabeled nucleic acid was then added. The antigens studied and their specific activity were tritiated polyadenylic acid ([^{3}H]poly A, 1 μCi/5.5 μg, single-stranded RNA), tritiated polyadenylic·polyuridylic acid ([^{3}H]poly A·poly U, 1 μCi/11.6 μg, double-stranded RNA), and tritiated native KB cell DNA ([^{3}H]nDNA, 0.23 μCi/μg). The amount of radiolabeled antigen added was: 7.74 ng/10 μl for [^{3}H]poly A, 91.6 mg/10 μl for [^{3}H]poly A·poly U, and 95.5 ng/10 μl for [^{3}H]nDNA. Sera were incubated with the radioactive antigen at 37°C for 30 min and then at 4°C for 18 h. The antigen-antibody mixture was then diluted with buffer and passed over a cellulose ester filter under suction. The filter was washed, dried, and placed in counting vials. The amount of radioactivity retained on the filter correlated directly with the concentration of antibody in the sample. The results are reported in nanograms of antigen bound per milliliter of serum. Values in excess of 2 SD above the normal mean for the 76 control family sera were: >73 ng/ml for [^{3}H]poly A, $> 16.4 \times 10^2$ ng/ml for [^{3}H]poly A·poly U, and $> 10.2 \times 10^2$ ng/ml for [^{3}H]-nDNA (Table I). Sera showing binding in excess of these values were considered positive for anti-nucleic acid antibodies. Multiple determinations of test sera as well as positive and negative control sera revealed that the coefficient of variation was consistently less than 10%.

Lymphocytotoxic antibodies were measured by the microdroplet method of Terasaki and McClelland (15). Sera were incubated with target cells from an average of 30 normal donors of varying HL-A phenotypes for 30 min at 15°C. Rabbit complement was then added with a further 3-h incubation (16). All sera were tested by utilizing the same batch of complement (Grand Island Biological Co., Berkley, Calif., lot A830124). Cell viability was measured by eosin dye exclusion. A serum had lymphocytotoxic activity if it killed 20% or more of the lymphocytes from 15 or more of the normal donors. All sera were tested against the same panel of donor target cells (8).

Fisher's exact test was used to calculate the association between the anti-nucleic acid antibodies and lymphocytotoxic antibodies, as well as the significance of anti-nucleic acid antibodies in the SLE family members compared to the controls (17).

RESULTS

Anti-nucleic acid antibodies. 5% of the total SLE family members and 6% of the household contacts had

TABLE I
Antibodies to [^{3}H]nDNA, [^{3}H]Poly A, [^{3}H]Poly A·Poly U in SLE and Control Family Members

	Number tested	Antigen bound		
		[^{3}H]nDNA	[^{3}H]Poly A	[^{3}H]Poly A·poly U
			ng/ml serum	
Total SLE family members	124	268.8 (12.8–19.7 × 10^2)	31.7 (0–368.7)	703.4 (52.4–36.5 × 10^2)
Household contacts	94	306.3 (12.8–19.7 × 10^2)	37.6 (2.1–368.7)	771.5 (65.4–36.5 × 10^2)
Nonhousehold contacts	30	218.7 (51.4–12.1 × 10^2)	11.9 (0–33.0)	527.7 (52.4–12.8 × 10^2)
SLE probands	28	25.5 × 10^2 (243.7–95.2 × 10^2)	218.8 (3.1–669.2)	15.8 × 10^2 (78.6–47.5 × 10^2)
Control family members	76	521 (89.9–15.4 × 10^2)	18.9 (1.0–73.3)	692.9 (13.0–25.5 × 10^2)

The values given are mean, with ranges in parentheses.

Table II

Percent of SLE and Control Family Members with Anti-Nucleic Acid and Lymphocytotoxic Antibodies

	Number tested	Anti-nDNA	Anti-RNA	Lymphocytotoxic
SLE family members	124	5 NS*	16 $P < 0.02$‡	57 $P < 0.0001$
Household contacts	94	6 NS	21 $P < 0.003$	68 $P < 0.0001$
Nonhousehold contacts	30	0 NS	0 NS	23 $P < 0.03$
SLE probands	28	68 $P < 0.0003$	82 $P < 0.0003$	82 $P < 0.0001$
Control family members	76	1	5	4

* NS, not significantly different from controls.
‡ Compared to control family members.

antibodies to nDNA (Table II). The incidence in the 28 SLE probands was 68% while the incidence in the 76 control families was 1%. Significance was reached only in the SLE patients. The occurrence in control and SLE family members was similar.

When the anti-RNA antibodies (anti-poly A, anti-poly A·poly U) were considered together, 16% of the total SLE family members, 21% of the close household contacts, and 82% of the SLE probands had these antibodies (Table II). 15 of 94 household contacts had antibodies to poly A ($P < 0.02$) while 9 had antibodies to poly A·poly U ($P > 0.1$). Only 5% of the control family members had anti-RNA antibodies. One control family member had both anti-poly A and anti-nDNA antibodies while three had antibodies to poly A·poly U. Thus, with respect to total anti-RNA antibodies, significant differences from controls were found for the SLE family members as a whole, the group with close household contact, and the SLE probands. These differences were due primarily to antibodies to poly A. The distribution of antibodies to poly A is demonstrated in Fig. 1.

Correlation of anti-nucleic acid and lymphocytotoxic antibodies. 57% of the 124 SLE family members had lymphocytotoxic antibodies (8). These antibodies were present in 68% of the household contacts, 23% of the nonhousehold contacts, 50% of the nonconsanguineous relatives, and 82% of the proband SLE patients (8). 4% of the control family members had these antibodies (Table II).

Correlation of anti-nDNA with lymphocytotoxic antibodies in the SLE probands (Table III) revealed 19 patients who had both antibodies and 5 who had neither. Four SLE patients had lymphocytotoxic antibodies without anti-nDNA while none had anti-nDNA without lymphocytotoxic antibodies ($P < 0.003$). In contrast, only 4 of the 124 family members had both anti-nDNA and lymphocytotoxic antibodies while 51 had neither ($P > 0.4$).

Total anti-RNA antibodies correlated with lympho-

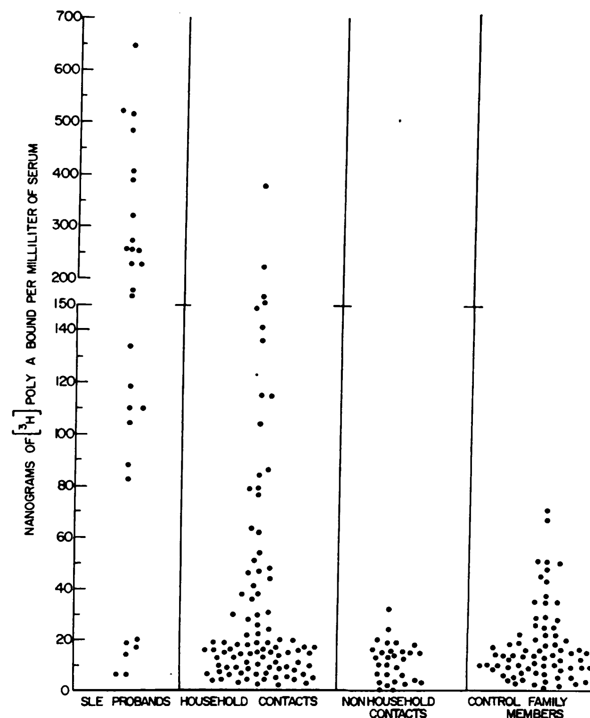

Figure 1 Distribution of antibodies to [^{3}H]poly A. The proband SLE patients have the highest levels. The nonhousehold contacts and controls have the lowest values while the household contacts are intermediate.

Table III

Correlation of Anti-Nucleic Acid and Lymphocytotoxic Antibodies in SLE Family Members

Lymphocytotoxic antibody	Anti-nDNA antibodies				Anti-RNA antibodies			
	SLE probands		Total SLE family members		SLE probands		Total SLE family members	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Positive	19	4	4	67	22	1	18	53
Negative	0	5	2	51	1	4	2	51
	$P < 0.003$		$P > 0.4$		$P < 0.0005$		$P < 0.003$	

cytotoxic antibodies in both the SLE probands and the 124 family members (Table III). Since no anti-nucleic acid antibodies were found in the nonhousehold contacts, no correlation was possible for this group. All correlations for the household contacts were similar to the SLE family members as a whole.

The specificity of the correlation of anti-RNA antibodies and lymphocytotoxic antibodies is demonstrated in Table IV. The 28 SLE probands included 22 patients with both anti-poly A (single-stranded RNA) and lymphocytotoxic antibodies, 5 with neither, and 1 who had lymphocytotoxic antibodies without anti-poly A. None of the proband SLE patients had anti-poly A without lymphocytotoxic antibodies. This association is highly significant ($P < 0.0003$). No association was found between anti-poly A and lymphocytotoxic antibodies in the household contacts. The correlation of anti-poly A·poly U (double-stranded RNA) with lymphocytotoxic antibodies was not significant for the SLE probands. On the other hand, the SLE family members as a whole and the household contacts more specifically had a significant correlation between anti-poly A·poly U and lymphocytotoxic antibodies ($P < 0.02$). In the family members as a whole, lymphocytotoxic antibody had a slightly higher association with anti-poly A·poly U than with anti-poly A.

In analyzing these data it became apparent that two variables were present, household contact and consanguinity. The incidence of anti-nucleic acid and lymphocytotoxic antibodies was then examined with each of the variables as a constant (Table V). When household contact was the constant, 7% of the consanguineous household contacts and 5% of the nonconsanguineous household contacts had anti-nDNA antibodies, a difference which is not significant. When anti-RNA antibodies were examined, 27% of the 74 consanguineous household contacts had these antibodies while none were present in nonconsanguineous household contacts ($P < 0.003$). Lymphocytotoxic antibodies, while present in both groups in an incidence higher than controls, were significantly higher in the consanguineous than in nonconsanguineous household contacts. When consanguinity was the constant, it can be seen from Table V that anti-nucleic acid antibodies were present only in the consanguineous household contacts. Lymphocytotoxic antibody was present in 73% of the 74 consanguineous household contacts and only 23% of the 30 consanguineous nonhousehold contacts ($P < 0.0001$). Again the incidence of lymphocytotoxic antibody in both of these groups was significantly higher than in controls (8).

DISCUSSION

A higher-than-expected incidence of anti-RNA antibodies (18, 19) has been demonstrated in family members of SLE patients. In the present study, the antibodies were absent in nonhousehold contacts but were present

Table IV

Correlation of Anti-RNA and Lymphocytotoxic Antibodies in SLE Family Members

Lymphocytotoxic antibody	Anti-poly A						Anti-poly A·poly U					
	SLE probands		Total SLE family members		Household contacts		SLE probands		Total SLE family members		Household contacts	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Positive	22	1	13	58	13	51	9	14	9	62	9	55
Negative	0	5	2	51	2	28	1	4	0	53	0	30
	$P < 0.0003$		$P < 0.03$		$0.1 > P > 0.05$		NS		$P < 0.02$		$P < 0.05$	

TABLE V
Comparison of Contact and Cosanguinity

	Number tested	Percent positive		
		Anti-nDNA	Anti-RNA	Lymphocytotoxic antibody
Household contacts				
Consanguineous	74	7	27	73
Nonconsanguineous	20	5	0	50
		NS*	$P < 0.003$	$P < 0.05$
Consanguineous relatives				
Household	74	7	27	73
Nonhousehold	30	0	0	23
		NS	$P < 0.003$	$P < 0.0001$

* No significant difference between the two groups.

in 21% of the household contacts, stressing the importance of possible environmental factors in the pathogenesis of SLE. A highly significant correlation was found between anti-double-stranded RNA and lymphocytotoxic antibodies in family members. The association between anti-single-stranded RNA (poly A) and lymphocytotoxic antibodies was less impressive. A different association was noted between these antibodies in the SLE probands. In the patients, lymphocytotoxic antibodies correlated with both anti-nDNA and anti-single-stranded RNA (poly A) but not with anti-double stranded RNA (poly A·poly U). These differences imply some selection at the level of the immunogen and suggest that immunization to nucleic acid is not a random process.

The incidence of anti-nucleic acid antibodies appears to be influenced by both contact with the proband and consanguinity in that anti-RNA antibodies are present only in consanguineous household contacts and not in nonconsanguineous household contacts nor in consanguineous relatives with infrequent proband contact. This relationship does not hold for anti-nDNA antibodies. However, the incidence of these antibodies is small and does not differ in control and SLE family members. Lymphocytotoxic antibodies also appear to be influenced by environmental and genetic factors. In this case, however, the genetic effect may be less critical for the antibodies were found in nonconsanguineous relatives.

Antibodies to nDNA, RNA, and lymphocytes have been previously reported in SLE (7, 11, 12, 20). Anti-nDNA and lymphocytotoxic antibodies have been shown to correlate with disease activity (20–22). The incidence of all three antibodies in our patients is comparable to that reported by others (7, 14, 19, 23). The incidence of anti-nDNA antibodies in SLE families is similar to other normal populations (14, 18) while that of anti-RNA antibodies is close to that reported in patients with rheumatic diseases other than SLE (14, 24). Antibodies to RNA found in SLE patients have greatest specificity for viral RNA (11, 24). Double-stranded RNA is found in greater amounts in tissues infected with RNA viruses, especially during the reproductive phase of the virus (25). The presence of antibodies to both lymphocyte surface antigens and to double-stranded RNA in apparently healthy SLE family members supports but does not prove the theory that a viral infection is an important factor in the pathogenesis of SLE (1, 26). The fact that the incidence of anti-nDNA antibodies was not elevated in SLE relatives suggests that the increase in anti-RNA and lymphocytotoxic antibodies is not due to a nonspecific hyperimmunity in these individuals. It is possible that these antibodies are indicators of exposure to an agent; moreover, they may have the same significance as a false positive serology that may precede the overt onset of disease in SLE patients (27). If family members have been exposed to a virus and have successfully handled the insult, a search for antibodies specific for this agent may be more fruitful in the consanguineous household contacts than in SLE patients who themselves may have developed a more complete loss of their immunologic control mechanisms (1, 2, 28).

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