Autoantibodies within Families of Patients with Systemic Lupus Erythematosus Are Not Directed Against the Same Nuclear Antigens

MICHIEL W. van der LINDEN, RUDI G.J. WESTENDORP, MAJIDA ZIDANE, LYDIE MEHEUS, and TOM W.J. HUIZINGA

ABSTRACT. ***Objective.*** The presence of antinuclear autoantibodies in systemic lupus erythematosus (SLE) is influenced by genetic factors. The presence of autoantibodies in healthy family members of patients has been reported. Our hypothesis was that autoantibodies are directed against the same antigens in first-degree family members of patients with SLE as in their patient relative.

Methods. Plasma was harvested from 50 patients with SLE, 154 unaffected first-degree family members, and 330 healthy controls. Presence of autoantibodies against 14 specific nuclear antigens was tested by the ELISA based line immunoassay INNO-LIA method.

Results. Seventy-four percent of patients, 32% of first-degree family members, and 1.5% of healthy controls had antibodies against any nuclear antigen. Most frequent autoantibodies in the patients were anti-histone and anti-SSA, whereas in the family members these were anti-RNP-C and anti-Topo-I/Scl. Presence and specificity of autoantibodies in family members were independent of the presence or absence of that autoantibody in their patient relative (chi-square $p > 0.1$ for all 14 antigens).

Conclusion. Autoantibodies in family members and their patient relatives are not directed against the same nuclear antigens. Thus a familial aspecific dysfunction of the B lymphocyte is the most likely explanation for autoantibody production in SLE. (J Rheumatol 2001;28:284–7)

Key Indexing Terms:
ANTINUCLEAR ANTIBODIES GENETICS SYSTEMIC LUPUS ERYTHEMATOSUS
AUTOIMMUNE DISEASES B-LYMPHOCYTES FAMILY STUDIES

Systemic lupus erythematosus (SLE) is characterized by the presence of autoantibodies directed against nuclear antigens. Some antinuclear autoantibodies (ANA) are associated with particular disease manifestations, e.g., anti-SSA antibodies with sicca syndrome, but most specific ANA are not[1,2]. Monozygotic, but not dizygotic twins have high concordance rates for SLE[3], indicating that genes play an important role in susceptibility for SLE. Presence of autoantibodies against nuclear antigens is more frequent among nonaffected family members of patients with SLE than in the general population. This familial aggregation suggests that presence of autoantibodies is under genetic control[4]. However, nongenetic factors may also be important in the generation of antigen specificity of autoantibodies, e.g., stochastic epitope selection[5]. Environmental factors may also be important given reports of ANA in unrelated individuals in the environment of the patients with SLE, and even in their household dogs[6]. We studied whether autoantibodies *within* families of patients with SLE are directed against one and the same specific antigen. The antigen specificity of ANA in patients was thus compared with the specificity of ANA in their first-degree family members.

From the Department of Clinical Epidemiology and the Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands, and Innogenetics NV, Ghent, Belgium.

M.W. van der Linden is supported by the Dutch Organization for Scientific Research, grant 904-61-110.

M.W. van der Linden, MD, Junior Researcher; R.G.J. Westendorp, MD, PhD, Associate Professor; M. Zidane, Department of Clinical Epidemiology; T.W.J. Huizinga, MD, PhD, Associate Professor, Department of Rheumatology, Leiden University Medical Center; L. Meheus, PhD, Research Associate, Innogenetics NV.

Address reprint requests to Dr. T.W.J. Huizinga, Department of Rheumatology, Building 1, C4-R, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands. E-mail: TWJHuizinga@rheumatology.azl.nl

Submitted March 16, 2000 revision accepted August 18, 2000.

MATERIALS AND METHODS

Fifty patients [age 39 ± 2 yrs (mean ± SE), 92% female] fulfilling at least 4 American College of Rheumatology criteria for SLE from single case families who attended the Rheumatology Department at Leiden University Medical Center from January 1, 1997, to October 1, 1998, were enrolled. The patients had disease activity of 2 (0–6) [median (interquartile range, IQR)] as measured with the SLE Disease Activity Index (SLEDAI)[7], and organ damage of 2 (1–4.5) as measured with the Systemic Lupus International Collaborating Clinics/ACR Damage Index[8]. Anti-DNA antibodies were present in 60% of these patients, and ANA measured with immunofluorescence using HEp-2 cells as a substrate were present in 94%. When the patients agreed to participate, all first-degree family members were also invited and interviewed for eligibility. Family members were asked about recent illness including any visits to their general health practitioner in the last 10 days, and about severe longstanding disease including

hospitalizations and specialized medical care in the last 10 years. A general history was taken. First-degree family members with autoimmune diseases were not included in the study. Medication was not allowed in the 24 hours prior to blood sampling. Altogether 154 healthy first-degree family members were enrolled [age 43 ± 1.4 yrs (mean ± SE), 57% female], yielding a family size of 4 (2–4) family members including the patient.

From each patient and family member 4 ml of citrated blood was centrifuged at 3000 g for 15 min and stored at –70°C. Semiquantitative titers of IgG autoantibodies against 14 specific purified nuclear antigens were determined in this plasma using the Line Immunoassay (INNO-LIA) method. This multiparameter assay has been validated in a multicenter evaluation and has greater sensitivity with equal specificity compared with conventional techniques of measuring ANA. Up to 98% agreement compared with combined conventional techniques (kappa values ranging from 0.54 to 0.9) were reported[9]. Recombinant antigens SSA/Ro52, SSA/Ro60, histone, SmB, SSB(La), Poly-dT, RNP-A, RNP-C, RNP-70K, ribosomal RNP, TopoI/Scl-70, CenP-B, and Jo-1 (HRS) were expressed as His fusion proteins in *Escherichia coli*, whereas SmD was additionally expressed as a His fusion protein in insect cells (*S. frugiperda* cells). Purified antigens were impregnated as parallel lines on a nylon test strip. Each test strip was incubated 1 h in a plastic trough containing 2 ml of standard antigen dilution to which 10 µl of plasma sample was added. Brown coloration of the test strip was interpreted as semiquantitative (+, ++, or +++) titers by 2 of us (MWL and MZ) independently. Whenever observations were not in agreement, a third observer decided.

Presence of any autoantibody was compared between first-degree family members and controls using binomial tests. Presence of specific autoantibodies as a dichotomous variable was compared between groups by chi-square (or Fisher's exact test where appropriate).

RESULTS

Seventy-four percent of the patients with SLE had autoantibodies against at least one antigen (Table 1). Of 37 patients with autoantibodies, 21 also had antibodies against DNA. One and a half percent (1.5%) of the control population (Caucasian North-European healthy blood donors) had antibodies. Of the first-degree family members, 32% had autoantibodies. Presence of autoantibodies in the first-degree relatives compared with controls resulted in p values < 0.01 in all cases. The spectrum of autoantibodies in the patients showed predominance of anti-histone and anti-SSA, whereas first-degree family members most frequently had autoantibodies against RNP-C and Topo-I/Scl70.

Next, the first-degree family members with autoantibodies against specific antigens were split up according to the presence or absence of autoantibodies against that antigen in their patient relative. In Table 2, 4 examples are given of presence of ANA in first-degree family members of patients with and without that ANA. Antibodies against SSA/RO52K were present in 2 of 41 first-degree family members of patients positive for anti-SSA/RO52K autoantibodies (5%). An equal proportion (5%) of the 123 first-degree family members of the patients without anti-SSA/RO52K autoantibodies were positive (by Fisher's exact test, p = 1.0). For anti-histone, anti-SmB, and anti-RNP-C autoantibodies, this comparison yielded similar proportions. Comparison of all 14 antigen specific autoantibodies between family members of patients with and without antibodies is presented in Table 3. Presence of a specific autoantibody was not different between family members of patients who were positive for that antigen and family members who were negative for that antigen. Moreover the presence of any of these 14 autoantibodies was similar in family members of patients with any autoantibody and family members without any autoantibodies (35% vs 26%; chi-square, p = 0.41).

Titers of autoantibodies in family members of patients with or without that autoantibody were also compared. No differences in autoantibody titer between family members of patients with or without that antibody were observed (Table 4).

DISCUSSION

This study confirms that the presence of autoantibodies is a

Table 1. Presence of antinuclear autoantibodies against 14 antigens in patients with SLE and their first-degree family members.

Antigen	Patients, n = 50 N (%)	First-degree Family Members, n = 154 N (%)
SSA/Ro52	12 (24)	8 (5)
SSA/Ro60	9 (18)	3 (2)
Histone	18 (36)	5 (3)
SmB	11 (22)	7 (5)
SmD	7 (14)	4 (3)
SSB(La)	9 (18)	6 (4)
Poly-dT	8 (16)	3 (2)
RNP-A	8 (16)	4 (3)
RNP-C	10 (20)	12 (8)
RNP-70K	2 (4)	0 (0)
Ribosomal RNP	2 (4)	0 (0)
Topo-I/Scl-70	2 (4)	9 (6)
Cenp-B	0 (0)	4 (3)
Jo-1 (HRS)	0 (0)	1 (1)
Any antigen	37 (74)	49 (32)

Table 2. Presence of autoantibodies in first-degree family members of SLE patients with and withoiut anti-SSA/Ro52K, anti-histone, anti-SmB, and anti-RNP-C autoantibodies.

Patients*, n = 50	Family Members, n = 154 With Specific Autoantibody (%)	Without Specific Autoantibody (%)
With anti-SSA/Ro52K	2 (5)	39 (95)
Without anti-SSA/Ro52K	6 (5)	107 (95)
With anti-histone	3 (5)	56 (95)
Without anti-histone	2 (2)	93 (98)
With anti-SmB	3 (9)	31 (91)
Without anti-SmB	4 (3)	116 (97)
With anti-RNP-C	4 (13)	26 (87)
Without anti-RNP-C	8 (6)	116 (94)

*Among the patients, 12 had autoantibodies against SSA/Ro52K whereas 38 did not. Eighteen patients had anti-histone, 32 did not; 11 had anti-SmB, 39 did not; and 10 had anti-RNP-C, 40 did not.

Table 3. Presence of autoantibodies in first-degree family members of SLE patients with and without autoantibodies.

Antigen*	Family Members Of Positive Patients Positive/Total (%)	Family Members Of Negative Patients Positive/Total (%)
SSA/Ro52K	2/41 (5)	6/113 (5)
SSA/Ro60K	1/22 (5)	2/132 (2)
Histone	3/59 (5)	2/95 (2)
SmB	3/34 (9)	4/120 (3)
SmD	0/19 (0)	4/135 (3)
SSB (La)	2/30 (7)	4/124 (3)
Poly-dT	2/28 (7)	1/126 (1)
RNP-A	1/18 (6)	3/136 (2)
RNP-C	4/30 (16)	8/124 (6)
RNP-70	0/4 (0)	0/150 (0)
Ribosomal RNP	0/4 (0)	0/150 (0)
Topo-isomerase	0/3 (0)	9/151 (6)
CenP-B	0/0 (0)	4/154 (3)
Jo-1	0/0 (0)	1/154 (1)
Any antigen	40/119 (34)	9/35 (26)

*All $p > 0.10$ (chi-square). The denominator of each proportion is the total number of first-degree family members of positive/negative patients, respectively. Note that the proportion of patients with autoantibodies is different from the various antigens, thus so is the number of family members of these positive patients.

Table 4. Semiquantitative titers of autoantibodies in positive first-degree relatives of patients with SLE.

Antigen	Relatives Of Positive Patients +	++	+++	Relatives Of Negative Patients +	++	+++
SSA/Ro52K	0	1	1	1	2	3
SSA/Ro60K	0	0	1	0	0	2
Histone	2	1	0	2	0	0
SmB	2	1	0	3	1	0
SmD	0	0	0	1	3	0
RNP-A	1	0	0	1	2	0
RNP-C	3	1	1	5	3	0
SSB	1	1	0	2	1	1
Poly-dT	2	0	0	1	0	0
Topo-isomerase	0	0	0	9	0	0
CenP-B	0	0	0	0	1	3
Jo-1	0	0	0	0	1	0

Semiquantitative titers of antinuclear autoantibodies were determined by visual inspection of brown coloring of the test strips. +: weak coloring, ++: moderate coloring, +++: strong coloring.

familial trait in SLE. However, the specificity of these antinuclear autoantibodies in family members was found to be independent of that in the patients. This indicates that the production of these autoantibodies is probably due to a familial, possibly genetic, intrinsic B lymphocyte defect. It may well be possible that patients with SLE and their family members have an increased frequency of circulating nuclear antigens[10]. Our results indicate, however, that antigen driven affinity maturation is not the main regulatory mechanism underlying genetic susceptibility for autoantibody production in SLE. Generation of these antigen-specific antibodies may be driven by environmental or stochastic, rather than genetic processes[5].

Increased presence of ANA in first-degree family members from simplex[1] and multiplex[11] SLE families has been reported before. In Arnett, *et al*[1], a higher proportion of family members of patients with anti-SSA/Ro autoantibodies was observed than in our study (21% and 27% in different subcohorts, vs 5% in the present study). Differences in assay might be responsible for this apparent discrepancy, since Arnett, *et al* employed an ELISA using bovine spleen and thymus as substrate, whereas INNO-LIA uses fusion proteins. Important in the Arnett, *et al* study is the finding that anti-SSA/RO (in combination with anti-SSB/La) autoantibodies were exclusively found in family members who were considered to have an "autoimmune trait" as defined by the presence of an autoimmune disease or other serum autoantibodies. In our study, first-degree family members with overt autoimmune or other disease were excluded. This is compatible with the study by Shoenfeld, *et al*, in which an association between autoantibodies, determined with ELISA, and overt disease could not be replicated[11].

Familial factors may be genetic in origin or otherwise inherited. The potential influence of environmental factors on presence and titer of autoantibodies is evident from reports of autoantibodies in unrelated individuals from the environment[12] and even pets of patients with SLE[6]. The reverse has also been reported (autoantibodies in humans sharing households with dogs suffering from canine lupus)[13]. This emphasizes the importance of comparing the first-degree family members with unrelated controls.

Factors that mediate aspecific production of antibodies by B lymphocytes include pleiotropic cytokines and growth factors such as interleukin 10 (IL-10), IL-6, and transforming growth factor-ß. Addition of recombinant IL-10 and IL-6 to peripheral blood lymphocytes of patients with SLE resulted in stronger expression of autoantibodies[14]. Administration of IL-10 to lupus-prone mice resulted in aggravation of their disease[15]. Moreover, administration of an anti-IL-10 antibody resulted in inhibition of the antibody production in peripheral blood mononuclear cells of patients with SLE that were infused in SCID mice[14]. In accord with these studies we and others have reported that familial production of IL-10 is increased in first-degree family members of patients with SLE[16-18]. Moreover, Grondal, *et al* showed that the endotoxin induced production of IL-10 in healthy spouses of patients with SLE is higher than that in controls[16]. These separate findings suggest that both genetic and environmental factors contribute to familial autoimmunity. This corroborates our interpretation that first-degree

family members of patients with SLE may have an intrinsic, familial B cell dysfunction.

ACKNOWLEDGMENT

B.A. de Jong is acknowledged for aid in reading the INNO-LIA test results.

REFERENCES

1. Arnett FC, Hamilton RG, Reveille JD, et al. Genetic studies of Ro (SS-A) and La (SS-B) autoantibodies in families with primary Sjögren syndrome. Arthritis Rheum 1982;32:413-9.
2. Cervera R, Khamashta M, Font J, et al. Systemic Lupus Erythematosus: clinical and immunologic patterns of disease expression in a cohort of 1,000 patients. Medicine 1993;72:113-24.
3. Deapen D. A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum 1992;35:311-8.
4. Miles S, Isenberg DA. A review of serologic abnormalities in relatives of SLE patients. Lupus 1993;2:145-50.
5. Eisenberg RA, Craven SY, Warren RW, Cohen PL. Stochastic control of anti-Sm autoantibodies in MRL/MP-lpr/lpr mice. J Clin Invest 1987;80:691-7.
6. Beaucher WN, Garman RH, Condemi JJ. Familial lupus erythematosus — antibodies to DNA in household dogs. N Engl J Med 1977;296:982-4.
7. Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH, Committee on Prognosis Studies in SLE. Derivation of the SLEDAI. A disease activity index for lupus patients. Arthritis Rheum 1992;35:630-40.
8. Gladman D, Ginzler E, Goldsmith C, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for systemic lupus erythematosus. Arthritis Rheum 1996;39:363-9.
9. Meheus L, van Venrooij WJ, Wiik A, et al. Multicenter validation of recombinant, natural and synthetic antigens used in a single multiparameter assay for the detection of specific anti-nuclear autoantibodies in connective tissue disorders. Clin Exp Rheumatol 1999;17:205-14.
10. Amoura Z, Piette JC, Chabre H, et al. Circulating plasma levels of nucleosomes in patients with systemic lupus erythematosus: correlation with serum antinucleosome antibody titers and absence of clear association with disease activity. Arthritis Rheum 1997;40:2217-25.
11. Shoenfeld Y, Slor H, Shafrir S, et al. Diversity and pattern of inheritance of autoantibodies in families with multiple cases of systemic lupus erythematosus. Ann Rheum Dis 1992;51:611-8.
12. Cabral AR, Alarcon-Segovia D. Autoantibodies in systemic lupus erythematosus. Curr Opin Rheumatol 1997;10:409-16.
13. Clair D, DeHoratius RJ, Wolfe J, Halliwell R. Autoantibodies in human contacts of SLE dogs. Arthritis Rheum 1980;23:251-3.
14. Llorente L, Zou W, Levy Y, et al. Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. J Exp Med 1995;181:839-44.
15. Ishida H, Muchamuel T, Sakaguchi S, Andrade S, Menon S, Howard M. Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. J Exp Med 1994;179:305-10.
16. Grondal G, Kristjansdottir H, Gunnlaugsdottir B, et al. Increased number of interleukin-10-producing cells in systemic lupus erythematosus patients and their first-degree relatives and spouses in Icelandic multicase families. Arthritis Rheum 1999;42:1649-54.
17. van der Linden MW, Westendorp RGJ, Sturk A, Bergman W, Huizinga TWJ. High production of interleukin-10 in first degree relatives of patients with generalized, but not cutaneous lupus erythematosus. J Invest Med 2000; (in press).
18. Llorente L, Richaud-Patin Y, Couderc J, et al. Dysregulation of interleukin-10 production in relatives of patients with systemic lupus erythematosus. Arthritis Rheum 1997;40:1429-35.