Lymphocytotoxic Antibodies in Family Members of Patients with Systemic Lupus Erythematosus

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ABSTRACT 57% of sera from 124 relatives of 28 patients with systemic lupus erythematosus (SLE) were found to have antibody directed against lymphocytes. The incidence in 60 members of 16 control families was 3%. Both consanguineous and nonconsanguineous relatives had the antibody in their sera. 68% of close household contacts of the SLE patients showed lymphocytotoxic antibody whereas only 23% of consanguineous relatives who had no household contact with the probands had this antibody. These data suggest that environmental factors may be important in the pathogenesis of SLE.

INTRODUCTION

The presence of lymphocytotoxic antibody has been described in patients with systemic lupus erythematosus (SLE)[1] (1, 2) and also the New Zealand black (NZB) mouse (3), an animal which often develops a disease closely resembling SLE. In both man (4) and the NZB mouse (3), lymphocytotoxic antibodies show relative specificity for thymus-derived or T lymphocytes; this phenomenon may be related to the alterations in T lymphocytes which accompany exacerbations of disease activity (5). The discovery of "viral-like" inclusions in tissues of SLE patients (6, 7) has stimulated speculation that this disease may be caused by a virus (8). Because lymphocytotoxic antibody has been described in several disease states of definite or probable viral origin (9–11), the current study was initiated to determine if lymphocytotoxic antibodies might serve as a marker of exposure to an environmental agent in families of patients with SLE.

METHODS

A total of 26 families which contained 28 patients with SLE were studied. All patients met the preliminary diagnostic criteria for SLE published by the American Rheumatism Association (12). One family contained a pair of identical twins, both of whom had active lupus nephritis. A second family contained two sisters, both of whom had active SLE. Sera from 124 family members were studied. First-degree relatives were defined as parents, children, and siblings of the proband while second-degree relatives were twice removed from the proband (i.e., grandparents, uncles, aunts, neices, and nephews). The family members were also divided into close household contacts and nonhousehold contacts. Close household contacts lived in the same house or had daily contact with the proband within the year before the onset and during the course of the proband's illness. Nonhousehold contacts included all relatives who did not live with the proband and had only casual contact with the proband during this time period. Sera were obtained from all of the household contacts and 21 of the 30 nonhousehold contacts within 4 wk of an acute exacerbation of the disease in the proband (13). Of 80 first-degree consanguineous relatives, 67 had close household contact while 13 did not have close household contact with the proband. 6 of 18 second-degree relatives had close household contact and 12 had no household contact with the proband. 20 SLE family members were related only by marriage. All 20 of these nonconsanguineous relatives had close household contact with the probands. Controls consisted of 60 sera obtained from members of 16 families matched with respect to age, sex, parity, ethnic background, socioeconomic status and geographic area (Table I). These controls included families of five medical students, two laboratory technicians, and three departmental secretaries. Three were families of neighbors or friends of medical students and three were families of physicians. Control family groups included only close household contacts. Neither the controls nor the SLE family members had signs or symptoms of SLE and none had an acute viral illness at the time of study. Both sets of sera were tested simultaneously against peripheral blood lymphocytes from three sources: (*a*) normal donors whose cells consisted of approximately

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[1] *Abbreviations used in this paper:* B lymphocyte, bone marrow-derived lymphocyte; CLL, chronic lymphocytic leukemia; HL-A, histocompatibility antigen; NZB, New Zealand black; SLE, systemic lupus erythematosus; T lymphocyte, thymus-derived lymphocyte.

80% T lymphocytes (14); (*b*) 15 proband SLE patients; (*c*) 6 patients with chronic lymphocytic leukemia (CLL) of the bone marrow-derived or B-lymphocyte type (15). Each serum was tested against lymphocytes from 15–60 normal individuals. The mean number of donors in the normal test panels was 30. The histocompatibility antigens (HL-A) of the donor lymphocytes were determined to assure that a wide spectrum of antigens were included in the panel. The microcytotoxicity method of Terasaki and McClelland (16) was used with 15°C incubations for 3 h after the addition of rabbit complement which was previously determined as optimal for the detection of lymphocytotoxins in patients with SLE (4). An individual test was considered positive if 20% or more of the lymphocytes from one donor were killed by a given serum. A strongly positive test had 80% or more of the donor lymphocytes killed by an individual serum. A serum was considered positive only if it killed 20% or more of the cells from at least half the donors in the panel. This criteria was chosen at the onset of the study before analysis of the data as one that would assure that we would be dealing with sera with broad cytotoxic activity. Negative sera, human serum albumin, and suitable controls for cell lysis alone consistently showed no more than 0–10% death of the target lymphocytes. All tests were read blind by an independent observer without knowledge of diagnosis or family status. Antinuclear antibodies were determined as previously described (17) and gamma globulin levels were estimated by cellulose acetate electrophoresis. Chi square analysis was used in all statistical comparisons between and within individual groups (18).

RESULTS

When the entire group of SLE family members was examined, 57% of 124 sera showed broad lymphocytotoxic antibody activity while only 3% of the 60 control sera demonstrated this phenomenon (Table II). No differences were noted between consanguineous and nonconsanguineous relatives of SLE patients. A considerably higher percentage of sera with lymphocytotoxic antibody activity (73%) was noted in the 74 consanguineous family members who had close household contact with the probands as compared to the 30 consanguineous relatives who did not have close household contact (23%) ($P < 0.0001$). The highest percentage of positive sera (82%) was found in the SLE probands. 45 of the 60 control sera killed cells of less than 10% of the donors in the test panel, while 11 of 28 sera from the SLE probands killed cells of over 90% of the normal donors (Table II). When taken as a whole, the distribution of the number of SLE family sera killing different percentages of the test panel was quite even. When broken down into consanguineous household and nonhousehold contacts, however, the household contact sera tended to react with more members of the panel than those from nonhousehold contacts ($P < 0.0001$).

The presence of lymphocytotoxic antibody activity in the SLE family members was also apparent when the distributions of the strength or actual degree of killing of all positive tests were compared (Table III). With the exception of the nonhousehold consanguineous relatives, a significantly higher percentage of strongly positive tests (greater than 80% killing) was found in SLE

TABLE I

Composition of the Two Groups Studied

	Number of individuals	
	SLE family members	Control family members
Age: <20 yr	34	16
≥20 yr	90	44
Sex: Male	62	30
Female (total)	62	30
(Multiparous)	(31)	(15)
Spanish American	62	34
Caucasian	54	23
Black American	8	3
Total	124	60

TABLE II

Percent Positive Lymphocytotoxicity of Sera against Normal Peripheral Blood Lymphocytes

	Number of sera	Percent positive sera	Number of sera with reactivity against normal peripheral blood lymphocytes									
			0–9*	10–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–100
Control family members	60	3	45	13	0	0	0	2	0	0	0	0
Total SLE family members	124	57‡	11	9	12	12	9	16	20	18	13	4
Consanguineous relatives	104	58‡	9	7	10	10	8	13	16	15	12	4
Household contacts	74	73‡	2	1	6	6	6	12	15	13	10	3
Nonhousehold contacts	30	23§‖	7	6	4	4	2	1	1	2	2	1
Nonconsanguineous relatives	20	50‡	2	2	2	2	2	3	3	3	1	0
Proband SLE patients	28	82‡	1	1	1	1	1	4	3	3	2	11

* Percent of donor lymphocyte panel killed by sera.
‡ Indicates a $P < 0.0001$ compared to controls.
§ Indicates a $P < 0.025$ when compared to controls.
‖ Indicates a $P < 0.0001$ compared to consanguineous household contacts.

TABLE III

Percent of Individual Cytotoxicity Tests Positive against Normal Peripheral Blood Lymphocytes

	Total number of tests	Donor cells killed in positive tests (Percent)				Total percent positive tests
		20–39	40–59	60–79	80–100	
Control family members	1,786	8*	4	1	0	13
Total SLE family members	3,690	25	17	9	9	60‡
Consanguineous relatives	3,106	20	16	10	9	55‡
Household contacts	2,214	19	18	9	10	56‡
Nonhousehold contacts	892	20	12	3	6	41‡§
Nonconsanguineous relatives	584	29	13	7	8	57‡
Proband SLE patients	800	21	13	13	33	80‡

* Percent of 1,786 tests in which 20–39% of the donor cells are killed.

‡ Indicates a $P < 0.0001$ when compared to controls.

§ Indicates a $P < 0.0001$ when compared to consanguineous household contacts.

family sera when compared to controls ($P < 0.0001$). A total of 60% of the tests between SLE family sera and normal donor lymphocytes were positive compared to 13% of the tests with control family sera.

Analysis of the 20 nonconsanguineous relatives of the probands revealed that 50% of these family members had lymphocytotoxic antibody. When the 1 female and 14 male spouses of the SLE probands were examined, 7 of 15 (47%) had lymphocytotoxic antibody. Moreover, in several situations where SLE probands lived with in-laws or with children from the spouse's previous marriage, substantial proportions (three of five) of these nonconsanguineous relatives showed lymphocytotoxic antibodies. When all consanguineous relatives were analyzed with respect to their relationship to the proband, 49 of the 68 (72%) first-degree relatives and 5 of 6 (83%) second-degree relatives with close household contact showed lymphocytotoxic antibody (Table IV). However, only 4 of 18 (22%) first-degree and 3 of 12 (25%) second-degree relatives without close household contact with the proband had lymphocytotoxins (Table IV). Five consanguineous relatives were studied on three separate occasions over a 12-mo period with no significant change in lymphocytotoxic activity.

Specificity of the lymphocytotoxic antibody in SLE family sera was examined with different target cells. If lymphocytes from the proband SLE patients were used, 9% of control sera and 73% of family sera were positive. Again no difference was noted between consanguineous and nonconsanguineous relatives. Sera from a given family showed no selectivity for the proband in that family. In contrast, only 4% of SLE family sera and 3% of control family sera were positive when CLL lymphocytes (95% or greater B lymphocytes) were used as target cells. Lymphocytotoxic antibody in family members or patients did not appear to show specificity for lymphocytes of any particular HL-A type. The incidence of both antinuclear antibody and gamma globulin elevation was 3% in the families of our SLE patients.

TABLE IV

Percent Positive Lymphocytotoxicity of Sera from Consanguineous Relatives

	Number of sera	Percent positive sera	
Control family members	60	3	
First-degree relatives	86	62	
Household contacts	68	72	$P < 0.001$
Nonhousehold contacts	18	22	
Second-degree relatives	18	44*	
Household contacts	6	83	$P < 0.02$
Nonhousehold contacts	12	25	

* Not significantly different than first-degree relatives.

DISCUSSION

The incidence of lymphocytotoxic antibody in a large group of relatives of patients with SLE was considerably higher than that found in control families. Lymphocytotoxic antibodies are rarely found in normal individuals (9) execpt after multiple pregnancies or transfusions (19). Moreover, the distribution of lymphocytotoxins far exceeded that of other serologic markers such as antinuclear antibodies or gamma globulin elevations recorded during previous family studies of SLE probands (20, 21).

As noted in Table I, the female control family members studied here showed a similar distribution of parity to those included in SLE families. No relatives studied in either SLE or control groups had received blood transfusions and none gave a history of recent upper-respiratory infection or viral illness. Therefore, a substantial increase in lymphocytotoxic antibody

among the SLE relatives appeared valid. Considerable proportions of SLE relatives showed strongly cytotoxic reactions where test sera killed 80-100% of test donor cells (Table III), whereas only occasional control family sera showed such strong reactions. SLE family members showed 13 times as many strongly cytotoxic reactions as control family sera tested.

Like those in SLE patients, the antibodies in their relatives appear to react with T lymphocytes. However, some sera which kill 100% of normal lymphocytes must also contain antibodies directed against B lymphocytes or common B- and T-cell antigens. It is apparent from the reaction patterns of sera form both patients (4) and their relatives that antigens occurring on normal lymphocytes are involved. These lymphocytotoxic antibodies do not have specificity for cells from patients with SLE. The slightly higher percentage of positive reactions with SLE family and control sera noted when SLE lymphocytes were used as targets can probably be explained by autosensitization of the cells with antilymphocyte antibodies in vitro (22). One explanation for this activity with normal cells is that new antigens appearing on SLE lymphocytes as the result of a virus infection (23) induce antibody against normal lymphocyte antigens as well as the new antigens (24). The suggestion that certain viruses may preferentially infect T lymphocytes (25) strengthens the hypothesis (8, 26) that a virus infection through its effect on T cells may be involved in the pathogenesis of SLE.

When individual SLE families were examined no clear genetic pattern of the inheritance of lymphocytotoxic antibody was recognized. Furthermore, the data showing an equal incidence of lymphocytotoxic antibodies in both consanguineous and nonconsanguineous household contacts of SLE probands are strong evidence against a purely genetic distribution. The 47% positive incidence of lymphocytotoxic antibody among spouses of SLE probands is pertinent in this regard. When these facts are considered together, the distribution of lymphocytotoxic antibodies in SLE families could be interpreted as evidence for a common environmental or infectious exposure.

Both environmental and genetic factors may be important in the production of disease in animal models of SLE. This is exemplified in both the dog and the NZB mouse models, where recent evidence suggests that both horizontal and vertical transmission of markers of the disease may occur (27, 28). In man, a clear genetic predisposition has not been found in twin studies (29, 30) nor has a definite relationship between HL-A markers and SLE been defined (31).

The data presented here do not eliminate the possibility of a genetic comp nent to SLE, since the antibody is also found in significantly greater proportion of blood relatives without household contact than in control family members. Even if a factor present in the environment is involved in the induction of lymphocytotoxic antibody, the ability to respond among individual patients or their relatives may be regulated by the immune response gene (32) linked in some fashion to other cell surface markers (33, 34). It is also conceivable that the lymphocytotoxic antibodies may be directed against immune response gene products or other cellular antigens not yet defined by current serologic techniques.

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