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Circulating antibodies that could be responsible for the suppressor thymus-derived (T)-cell dysfunction in active systemic lupus erythematosus (SLE) were investigated. Sera from 14 active and inactive SLE patients were compared with a pool of 22 normal sera. All sera were adsorbed with a pool of normal platelets to exclude antihistocompatibility leukocyte antigen antibodies; with AB erythrocytes to exclude isohemagglutinins; and with a pool of normal bone marrow-derived (B) lymphocytes, monocytes, and neutrophils to deplete anti-B-cell antibodies, Fc-receptor antibodies, and antibodies directed against neutrophils or monocytes. Sera from active SLE patients were capable of inhibiting the activation of normal, blood lymphocytes by concanavalin A to become suppressor cells. The latter were assayed by coculturing the concanavalin A-activated cells with autologous lymphocytes, which were then activated with either phytohemagglutinin for proliferative response or with pokeweed mitogen for B-cell immunoglobulin (Ig) synthesis and secretion. Specific incorporation of cultures with phytohemagglutinin showed a value of 67±13 (mean±SD) for suppressor cells treated with adsorbed, active SLE sera. This value was significantly different ($P < 0.001$) from that of cells treated with the inactive SLE sera or with the pool of normal sera. Similar findings were seen with respect to the B-cell target parameters. Cytoplasmic Ig and IgG in supernates of cultures with pokeweed mitogen showed values of 17±5% and 717±134 ng/culture, respectively, for suppressor cells […]

Suppressor-Cell Antibody in Systemic Lupus Erythematosus

POSSIBLE MECHANISM FOR SUPPRESSOR-CELL DYSFUNCTION

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ABSTRACT Circulating antibodies that could be responsible for the suppressor thymus-derived (T)-cell dysfunction in active systemic lupus erythematosus (SLE) were investigated. Sera from 14 active and inactive SLE patients were compared with a pool of 22 normal sera. All sera were adsorbed with a pool of normal platelets to exclude antihistocompatibility leukocyte antigen antibodies; with AB erythrocytes to exclude isohemagglutinins; and with a pool of normal bone marrow-derived (B) lymphocytes, monocytes, and neutrophils to deplete anti-B-cell antibodies, Fc-receptor antibodies, and antibodies directed against neutrophils or monocytes. Sera from active SLE patients were capable of inhibiting the activation of normal, blood lymphocytes by concanavalin A to become suppressor cells. The latter were assayed by coculturing the concanavalin A-activated cells with autologous lymphocytes, which were then activated with either phytohemagglutinin for proliferative response or with pokeweed mitogen for B-cell immunoglobulin (Ig) synthesis and secretion. Specific incorporation of cultures with phytohemagglutinin showed a value of 67±13 (mean±SD) for suppressor cells treated with adsorbed, active SLE sera. This value was significantly different ($P < 0.001$) from that of cells treated with the inactive SLE sera or with the pool of normal sera. Similar findings were seen with respect to the B-cell target parameters. Cytoplasmic Ig and IgG in supernates of cultures with pokeweed mitogen showed values of 17±5% and 717 ±134 ng/culture, respectively, for suppressor cells treated with the adsorbed, active SLE sera. This was significantly different from those treated with the inactive SLE sera or with the pool of normal sera. The antisuppressor-cell factor was shown to be IgG, complement independent, not cytotoxic, active at 37°C and at room temperature, but not at 4°C, and adsorbable with T cells.

Suppressor T-cell antibody in sera of active SLE patients could be responsible for the observed suppressor T-cell dysfunction seen in active SLE. The mechanisms responsible for the induction of the anti-suppressor-cell antibody are unknown.

INTRODUCTION

Suppressor-cell dysfunction was postulated (1) and shown (2–6) to play a key role in the expansion and hyperactivity of the autoreactive clones in systemic lupus erythematosus (SLE).[1] It has recently been shown by us that suppressor-cell dysfunction in active SLE is limited to the thymus-derived (T)-cell subpopulation, as shown by their failure to be activated by concanavalin A (Con A) (6).

Lymphocytotoxic autoantibodies were shown in sera of SLE patients by several investigators (7–10). Some of them were shown to be specific for T cells (8, 9) and a T-cell subpopulation (10). In NZB/W mice, which simulate human SLE in several ways, natural thymocytotoxic antibody (NTA) was detected in sera of these animals early in life, before the detection of suppressor-cell deficiency (11, 12). The antibody was shown to have antisuppressor T-cell activity (12, 13)

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[1] *Abbreviations used in this paper*: Con A, concanavalin A; NTA, natural thymocytotoxic antibody; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SI, specific incorporation; SLE, systemic lupus erythematosus.

and was implicated in the pathogenesis of the autoimmune state in these mice (11–13).

In this report we have shown that the sera of active SLE patients are capable of inhibiting the in vitro suppressor potential of Con A-activated, blood mononuclear cells of normal donors. We propose that the antisuppressor-cell antibody plays a key role in the induction and(or) the maintenance of the suppressor-cell dysfunction that leads to the expansion and hyperactivity of the autoreactive clones in active SLE.

METHODS

Patients. 14 patients with documented SLE were studied. Details of the main clinical and serological features of these patients have been published (6). Patients were studied when their disease was active and again during clinical remission. Disease activity was determined by a rise in sedimentation rate, fever, and increased symptoms, together with a drop in serum complement and(or) increased serum antinative DNA antibody. The controls were 22 normal, healthy laboratory or medical personnel that were roughly age-matched to the SLE patients.

Adsorption of the various sera. The individual lupus sera or the pool of control sera were decomplemented by heating at 56°C for 30 min. They were then adsorbed by pooled platelets, AB erythrocytes, neutrophils, and blood bone marrow-derived (B) cells and monocytes. The pool was obtained from 12 different, normal, healthy volunteers. Platelets were harvested from plasma after centrifugation of heparinized blood at 720 *g*. Erythrocytes and neutrophils were collected as pellets after fractionation of heparinized blood by the standard Ficoll (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.)-Hypaque (Winthrop Laboratories, New York) technique (14). Blood B cells and monocytes were harvested from the interphase layer by the E-rosette centrifugation method as described by us (15). Adsorption of sera by the pooled cells was done at room temperature for 2 h and then at 4°C overnight. For each 1-ml serum, 1×10^8 of each of the cell types was used. The adsorbed sera were collected by centrifugation at 2,000 *g* at 4°C for 30 min and then sterilized by filtration through 0.22-micropore size Millipore filters (Millipore Corp., Bedford, Mass.).

Treatment of normal, blood mononuclear cells with the adsorbed sera. Blood mononuclear cells obtained from normal volunteers by the standard Ficoll-Hypaque method (14) were incubated with the adsorbed SLE or control sera using various doses, periods, and temperatures, with or without guinea pig complement. The cells were then washed thrice in RPMI 1640 with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.), incubated for 24 h with or without 20 μg Con A (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden), washed thrice, and then cocultured with fresh, autologous, blood mononuclear cells, to which 10 μg phytohemagglutinin (PHA) or 100 μg pokeweed mitogen (PWM) was added for 3 or 7 d, respectively. The culture conditions and the evaluation of proliferative response and B-cell Ig synthesis and secretion were performed by previously described methods (2, 6, 15).

Evaluation of the Ig nature of the antisuppressor-cell antibody. The adsorbed sera were treated with activated Sepharose 4B (Pharmacia Fine Chemicals, Inc.) columns, to which heavy chain specific anti-human IgG, IgM, or IgA antisera were bound. Depletion of the various Ig classes was confirmed by immunoelectrophoresis. The adsorbed, Ig-depleted sera were then tested for antisuppressor-cell activity as outlined above.

The cytotoxicity assay was performed by the standard 1% trypan blue exclusion method (15), and the statistical analysis was done with the Student's *t* test.

RESULTS

Preliminary experiments were performed to characterize the optimal conditions for inhibiting the Con A activation of normal, blood mononuclear cells. It was found that 0.1 ml of the adsorbed sera added to 1×10^6 cells for 45 min at 37°C or room temperature was optimal for the inhibition. Adding 0.1–0.5 ml guinea pig complement to the culture did not enhance the inhibitory activity of the sera, nor did it result in cell killing as detected by the dye exclusion method. Therefore, in the experiments shown in Tables I–III, 0.1 ml of the various adsorbed sera were added to 1×10^6 normal, blood mononuclear cells suspended in 1 ml RPMI-1640 and incubated at room temperature for 45 min in the absence of complement.

Table I shows that sera from the active SLE patients eliminated the suppressive effect of Con A-activated cells in the autologous PHA-stimulated cultures. This is demonstrated by a specific incorporation (SI) ratio of 67±13 (mean±SD) which is not significantly different from the SI of cultures not activated by Con A (footnote, Table I). The SI values of cultures treated with the active SLE sera were significantly different from those treated with the inactive SLE sera or with the pool from the control sera ($P < 0.001$). Table I also shows that there were no significant differences of the background counts of the various groups.

TABLE I

Effects of Treatment of Normal, Blood Mononuclear Cells with Controls or SLE-Adsorbed Sera on the Proliferative Responses to PHA

Source of adsorbed sera	No. of sera tested	Background cpm	SI*	Percent suppression‡
		mean±SD	*mean±SD*	*mean %*
Controls	22 (Pool)	207±97	11±9	65
Inactive SLE	14	278±142	19±14§	74§
Active SLE	14	304±194	67±13‖	7‖

* SI = cpm in presence of PHA/background cpm in absence of PHA.

‡ Percent suppression = 1 − SI of cultures treated with Con A/SI of cultures not treated with Con A × 100. Cultures not treated with Con A gave background cpm of 259±79 and SI of 72±18.

§ $P > 0.3$ when compared with values of control-adsorbed sera.

‖ $P < 0.001$ when compared with values of control or inactive SLE-adsorbed sera.

TABLE II

Effects of Treatment of Normal, Blood Mononuclear Cells with Controls or SLE-Adsorbed Sera on PWM-Induced Ig Synthesis and Secretion

Source of adsorbed sera	No. of sera tested	Cytoplasmic Ig		IgG secretion	
		% positive	*% suppression**	*ng/culture*	*% suppression**
Controls	22 (Pool)	7±3	65	162±37	77
Inactive SLE	14	10±4‡	50	204±72‡	71
Active SLE	14	17±5§	15	717±134‖	4

Cultures not treated with Con A gave 20±6% positive cells and 694±204 ng/culture.
* Percent suppression = 1 − Ig parameter of cultures treated with Con A/Ig parameter of cultures not treated with Con A × 100.
‡ P not significantly different from control adsorbed sera values.
§ $P < 0.05$ when compared with control adsorbed sera values.
‖ $P < 0.001$ when compared with control or inactive SLE-adsorbed sera values.

Table II shows that sera from the active SLE patients have also inhibited the suppressive effects of Con A-activated cells on PWM-stimulated autologous B-cell cytoplasmic Ig and IgG secretion. This is shown in Table II by a percent positive cytoplasmic Ig of 17±5% and an IgG secretion of 717±134 ng/culture. These values are not significantly different from those of cultures not activated by Con A (footnote, Table II) and significantly different from those treated with the inactive SLE sera or with the pool from the control sera.

Depletion of IgG, but not of IgM or IgA from five active SLE sera that were tested eliminated the inhibitory effects on the Con A-activated cells (Table III). This would indicate the IgG nature of the inhibitory factor in the active SLE sera.

Adsorption of the five active SLE sera with normal, pool T cells eliminated the inhibitory effects of the Con A-activated cells (Table III).

DISCUSSION

In this report we have shown that sera from active SLE patients contain IgG antibodies that can prevent the activation of normal, blood mononuclear cells to become suppressor cells. The antibody could not be detected in the sera of the same SLE patients when their disease became inactive and is probably the main factor responsible for the T-cell dysfunction in active SLE. It has been shown by Glinski et al. that patients with active SLE had a decrease in a subpopulation of T cells upon separation on a discontinuous Ficoll gradient (10). The reduction was shown to be a result of an IgG antibody that is capable of depleting the same subpopulation from normal, blood lymphocytes. Recently, it has been shown that blood from active juvenile rheumatoid arthritis patients have fewer regulatory subsets of T cells and higher numbers of cells secreting Ig (16). Sera from the same patients have antibodies that react with normal T cells. The latter subpopulation responds to Con A in vitro, but cannot enhance PWM-stimulated B-cell Ig production (17).

Sera with high titer NTA activity in NZB/W mice were shown to recognize a subpopulation of T cells and decrease the response of normal spleen cells to Con A as compared with PHA (11). Repeated administration of NTA to 3- to 5-d-old NZB/W mice resulted in the acceleration of loss of suppressor T-cell function, shortened life span, and increase in capacity to induce graft vs. host disease (12). Shirai et al. have shown that NTA at high dilutions can selectively decrease suppressor T cells (13). Analysis by fluorescence-activated cell sorter revealed that mouse suppressor T cells have high NTA-reactive antigen (13) and that human regulatory T cells bind to sera from

TABLE III

Effects of Depletion of Various Immunoglobulins or of Anti-T-Cell Antibody from the Adsorbed, Active SLE Sera on Their Inhibitory Activity of Suppressor Cells

Active SLE sera depletion of*	PHA response	Percent suppression of‡ cytoplasmic Ig-positive cells	IgG in supernates
	% suppression	*% suppression*	*% suppression*
None	5±4	7±5	9±4
IgG	72±17§	69±22§	57±14§
IgM	9±3	4±3	8±3
IgA	7±4	6±4	10±6
Anti-T cell	84±12§	75±14§	66±19§

* See Methods for details of the depletion. Five different sera were tested.
‡ See footnotes, Tables I and II.
§ $P < 0.001$ when compared with results of sera not depleted (first line).

active juvenile rheumatoid arthritis patients (16, 17). These studies, ours and those recently published by Twomey et al. describing antibody effects on Con A-induced suppression in SLE patients, indicate that these antibodies could be responsible for the preferential loss of suppressor T cells that would lead to the various autoimmune abnormalities (16, 18, 19).

The mechanisms responsible for the production of the antisuppressor-cell antibody in active SLE are unknown. We can postulate that genetic factors combined with viral infections with specificity to receptors on the suppressor-cell subpopulation could result in transient changes in the antigenicity of these cells. This will result in a break of self-tolerance and the formation of autoantisuppressor-cell antibody, suppressor-cell loss and(or) dysfunction, and the subsequent initiation or activation of the autoimmune state.

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