Suppressor-Cell Dysfunction in Systemic Lupus Erythematosus

CELLS INVOLVED AND IN VITRO CORRECTION

AKIRA SAGAWA and NABIH I. ABDOU, *Department of Medicine, Section of Allergy, Clinical Immunology, and Rheumatology, University of Kansas, Kansas City, Kansas 66103, and Veterans Administration Hospital, Kansas City, Missouri 64128*

ABSTRACT To characterize the cell(s) responsible for the suppressor-cell dysfunction in active systemic lupus erythematosus (SLE), we fractionated blood mononuclear cells into thymus-derived (T), bone marrow-derived (B), and monocyte-depleted populations. Various cell populations from active SLE, inactive SLE, or normals, were activated with Concanavalin A, washed, and then co-cultured with active SLE cells. Soluble immune response suppressor (SIRS) from culture supernates of the activated cells was also used for the possible correction of the suppressor-cell dysfunction. Suppression was tested by enumerating DNA-binding cells by radioautography and by quantitating anti-DNA antibody in culture supernates by radioimmunoassay; and immunoglobulin was tested in cells and supernates by the immunofluorescence and the immunofluor techniques, respectively. Except for the numbers of DNA-binding cells, which were not suppressed, all the other three parameters in co-cultures with cells from active SLE patients were suppressed by Concanavalin A-activated cells ($P < 0.001$), or by SIRS ($P < 0.05$) from normals or inactive SLE patients. Concanavalin A-activated autologous or allogeneic active SLE cells and nonactivated cells from active or inactive SLE failed to suppress the various B-cell functions. Nonactivated normal cells suppressed levels of anti-DNA and immunoglobulin in supernates ($P < 0.05$).

In characterizing the cells responsible for the suppressor dysfunction, it was clear from the results that T cells responsive to Concanavalin A activation are deficient in active SLE and fail to generate SIRS. On the other hand, monocytes from active SLE patients are responsive to signals from the activated T cells of normals or inactive SLE donors. Because SIRS suppresses active SLE cells in vitro, it might be considered therapeutically for the in vivo modulation of SLE.

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INTRODUCTION

In normal states there is ample evidence which indicates that autoreactive cell clones (DNA-binding or thyroglobulin-binding cells) are present in the peripheral blood in small numbers (1). In autoimmune states, expansion of these autoreactive clones has been postulated (2, 3) and shown (4) to be a result of abnormal immunoregulation secondary to suppressor-cell dysfunction.

In active states of human systemic lupus erythematosus (SLE)[1] we demonstrated suppressor-cell dysfunction, which was reversible and became normal when the disease remitted (4). In the NZB/W mouse model of SLE, suppressor thymus-derived (T)-cell deficiency was demonstrated early in life and became progressive during the short life span of the animals (5). Though the animals were deficient in suppressor cells, they could respond to suppressor signals from normal mice (6).

Mouse (7, 8) and human (9) suppressor T cells can be activated by their in vitro incubation with concanavalin A (Con A). Activated suppressor cells secrete a soluble factor called soluble immune response suppressor (SIRS) (10). The latter was demonstrated to suppress various in vitro immune parameters (11) and was recently demonstrated to be capable of correcting the various autoimmune phenomena in the NZB/W mouse when injected repeatedly before the onset of the disease (6).

[1] *Abbreviations used in this paper*: Con A, concanavalin A; SIRS, soluble immune response suppressor; SLE, systemic lupus erythematosus.

In this study we have attempted to identify the cell(s) in human SLE responsible for the suppressor-cell dysfunction and to correct it in vitro by coculturing SLE cells with Con A-activated SLE or normal cells, or by SIRS obtained from SLE or normal cells. The results clearly demonstrate that both T cells and monocytes are required for suppression, and that the former initiates suppression upon Con A activation by secreting SIRS that mediates suppression to monocytes. In active SLE, the defect is predominantly at the level of the suppressor T cell. Anti-DNA secretion, immunoglobulin synthesis, and secretion by the active lupus cells could be inhibited by Con A-activated cells or SIRS from normal or inactive SLE donors. Activated cells or SIRS from active SLE donors failed to inhibit the various parameters. The numbers of DNA-binding cells were not affected by activated cells or SIRS from either normals or the SLE patients.

METHODS

Patients and immunologic workup. 14 patients with documented SLE, and who satisfied the diagnostic criteria of the American Rheumatism Association for SLE (12) were studied. This study was approved by the institution's human experimentation committee and informed consents were obtained from all the patients who entered the study. Details of the main clinical and serological features are shown in Table I. Seven patients were studied twice, 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 or increase in serum anti-native DNA antibody. None of the patients were on cytotoxic drugs. Prednisone dose received by the patients at the time of study is shown in Table I, with a mean of 35 and 10 mg/day in the active and inactive groups, respectively. Two control groups were studied, patients and normals. One patient group included four cases of biopsy-proven polyarteritis nodosa on a mean dose of 37.5 mg oral prednisone. These four patients had active disease as manifested by increased erythrocyte sedi-

TABLE I
Patient Material: Clinical and Serological Parameters

Patient	Age Sex	Duration of disease	Disease activity	Main clinical features	Serum C_3*	Serum %^{3}H-DNA binding‡	ESR§	R_x prednisone
		yr			*mg/100 ml*		*mm/h*	*mg/day*
1	24 F	4	Active	Arthritis, nephritis	56	72	62	60
			Inactive	Rash	127	16	19	5
2	32 F	6	Active	Arthritis, rash	94	54	43	0
			Inactive	Asymptomatic	102	21	12	0
3	17 F	3	Active	Serositis, arthritis	82	69	89	25
			Inactive	Asymptomatic	117	14	20	0
4	47 F	9	Active	Nephritis, Raynaud's	62	79	72	40
			Inactive	Asymptomatic	89	27	17	20
5	42 F	2	Active	Rash, alopecia	120	17	39	15
			Inactive	Asymptomatic	114	19	20	15
6	37 F	1	Active	Bullous rash, alopecia	127	24	94	120
			Inactive	Asymptomatic	141	15	12	15
7	24 F	4	Active	Arthritis, rash	119	14	34	10
			Inactive	Asymptomatic	111	17	21	5
8	27 F	2	Active	Neuropsychiatric, rash	104	14	39	60
9	35 F	3	Active	Serositis, rash	78	39	61	20
10	22 F	3	Active	Arthritis, rash	127	32	34	20
11	22 F	1	Active	Neuropsychiatric, rash	115	41	68	40
12	29 F	2	Active	Rash, serositis	94	61	27	15
13	37 M	2	Active	Rash, myalgia	79	29	39	25
14	24 F	3	Active	Nephritis, arthritis	69	56	54	40

* Normal values 82–138 mg/100 ml.
‡ The percent of 25 ng ^{3}H-DNA bound by 25 μl serum. Normal <20%.
§ Erythrocyte sedimentation rate by the Westergren method.

mentation rate (44±17 mm/h) and clinical evaluation. The other patient group included four patients with severe steroid-dependent bronchial asthma on a mean dose of 12.5 mg oral prednisone. 21 healthy laboratory or hospital personnel who were sex matched and roughly age matched constituted the normal control group.

Complement (C_3) quantitative assay was done by the radial-immunodiffusion technique with commercial immunoplates (Meloy Laboratories Inc., Springfield, Va.). The binding of sera to native DNA was performed by the Millipore filter radioimmunoassay (Millipore Corporation, Bedford, Mass.) (13) with ^{3}H-DNA (Electro-Nucleonics Inc. Separation & Analytical Sys. Div., Fairfield, N. J., lot no. 1452).

Generation of suppressor cells and SIRS. Mononuclear cells were separated from heparinized blood by the standard Ficoll-Hypaque technique (Pharmacia Fine Chemicals Inc.) (14). In seven experiments, mononuclear cells were fractionated into enriched T-(>95%), enriched B-(>87%), and monocyte-depleted (<2%) populations as described by us earlier (4, 15). Briefly, B- and T-cell fractionation was achieved by the E-rosette centrifugation method (4). Monocytes were depleted by allowing mononuclear cells to phagocytose iron filings followed by magnet depletion (15). In five experiments, monocytes were depleted by allowing them to stick to a plastic surface on a Petri dish (no. 3002, Falcon Plastics, Oxnard, Calif.) for 2 h at 37°C. The unattached cells were washed gently and decanted. The attached cells were then recovered by scraping with a rubber spatula. The purity of the various fractions was tested by the E-rosette technique for T cells (4), surface membrane immunoglobulin for B-cells (4), and nonspecific esterase for monocytes (15).

Various cell fractions and unfractionated cells were washed three times and then incubated for 1 h at 37°C in medium RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) in the absence of serum and then washed three times. This allowed shedding and removal of any passively adsorbed antibody (4). In four experiments, 1 ml of the last wash of cells from the active SLE patients was tested for the presence of immunoglobulin (Ig)G and anti-DNA antibodies; none was detected. 2×10^6 mononuclear cells were cultured in tubes (no. 2001, Falcon Plastics) which contained 1 ml medium RPMI-1640, 20% fetal calf serum (Microbiological Associates, Walkersville, Md.), and various doses (2, 20, and 60 μg) of Con A. Cells were cultured for 48 h at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Control tubes to which no Con A was added were processed the same way. After the incubation period, both cells and supernates were harvested. The cells were washed three times and then tested for suppressor activity. In five experiments, separate tubes were treated with ^{3}H-Con A (New England Nuclear, Boston, Mass.), and the amount of radioactivity carried by the cells after the 48-h culture and washing procedure was calculated and found to be <3.4% (<68 ng Con A) of the amount added at the initiation of the culture. 60 ng of Con A was added to the non-Con A-activated control cells (to control for the residual Con A left on the Con A-activated cells).

The culture supernates were adsorbed three times with equal volumes of packed Sephadex G-50 (Pharmacia Fine Chemicals, Inc.) for 1 h at 4°C, sterilized, and stored at −70°C. The volume used for the suppressor assay was expressed as a fraction of 1 ml of the SIRS raised by 2×10^6 cells. Control supernates raised in the absence of Con A were handled and adsorbed in an identical fashion to SIRS. The amount of Con A remaining in the supernate after repeated adsorption with Sephadex was monitored in five experiments with ^{3}H-Con A and was found to be <2.2% (<44 ng Con A) of the added Con A. 40 ng was added to the control supernates to control for the residual Con A in the SIRS.

Testing for suppressor activity of cells or SIRS. 1×10^6 Con A-activated or non-Con A-activated washed cells, or 0.1–1.0 ml SIRS obtained from Con A-activated or non-Con A-activated (control) cultures were used. These were co-cultured with 1×10^6 fresh, autologous or allogeneic mononuclear cells obtained from active SLE patients or from controls. The culture conditions were as described by us earlier (4). Briefly, 2×10^6 cells/tube were cultured in 2 ml medium RPMI-1640 and 15% fetal calf serum to which was added 100 μg pokeweed mitogen (Grand Island Biological Co.). Controls were 1×10^6 cells of each cell type cultured alone in the presence of pokeweed mitogen. The tubes were incubated for 7 days and then cell supernates were tested for ^{3}H-DNA-binding by the Millipore filter assay (13) as described by us earlier (4) and for immunoglobulin content with immunofluor kits (Bio-Rad Laboratories, Richmond, Calif.).

In all the assays performed for DNA or immunoglobulin in either cells or supernates, the values obtained from cell cultures of suppressor (Con A- or non-Con A-activated) cell types alone were subtracted from values of the cocultures.

DNA binding to cell culture supernatants. Binding to native DNA was performed by the Millipore filter assay (13) with tritiated double-stranded DNA. Briefly, culture supernates of 1 ml each, were concentrated 10 times by dialysis (Minicon A-75, Amicon Corp., Scientific Sys. Div., Lexington, Mass.). 5 μl of the concentrated supernate was incubated with 0.1 ml of tritiated DNA (lot no. 1452, Electro-Nucleonics Inc.) at 37°C for 30 min. Before use, the tritiated DNA, 1.0 μg/ml, diluted in sodium chloride citrate buffer, pH 8.0, was filtered through a 0.45-μm pore size Millipore filter. The specific activity of ^{3}H-DNA was 0.129 μCi/μg, and the source of DNA was KB human cell line. After incubation of the sample with ^{3}H-DNA, the mixture was filtered through similar filters at a rate of 1.0 ml/min. Filters were washed with 4 ml of buffer, dried, and placed in scintillation vials for counting. The percentage of binding was calculated by the formula: (counts per minute of filters of tested material/counts per minute of 0.1 μg of the filtered ^{3}H-DNA) × 100.

^{3}H-DNA binding to cells and radioautography. To each 2×10^6 washed mononuclear cell, 0.2 ml (200 ng) of the tritiated DNA was added and incubated at 4°C for 1 h under continuous rotation. The ^{3}H-DNA was the same as that used for the supernates. Cells were then washed three times in medium RPMI-1640 which contained 10% fetal calf serum. The cells were then processed for radioautography (16). Slides were developed for 3 wk. 10 slides were made from each preparation and 1,000 cells were counted on each slide. Results are expressed as positive cells per 10^5 cells counted.

Testing for cytoplasmic immunoglobulin. The technique used was described in detail by us earlier (4). We tested the cells by fluorescein isothiocyanate-conjugated antihuman polyvalent immunoglobulin antisera (Behring Diagnostics, American Hoechst Corp., Somerville, N. J.).

Testing for immunoglobulin (Ig) in supernates. The immunofluor kits for IgG and IgM were used as described by the manufacturer. IgM kits were found insensitive for the assay and therefore the results shown are those for IgG.

Statistical analysis. The Student's t test was used and P values < 0.05 were considered statistically significant.

RESULTS

Effects of Con A-activated cells. In preliminary experiments in which various doses of Con A were used to activate suppressor cells, we found that under the culture conditions stated above, 2 and 20 μg of Con A were optimal. 60 μg of Con A was toxic to the

cells in the 48-h culture. Therefore in all the experiments stated in the results, we used 20 μg of Con A to activate suppressor cells and to generate SIRS.

Generation of suppressor cells from various donors. As can be seen in Table II, activated mononuclear cells from either autologous or allogeneic active SLE patients failed to suppress either ^{3}H-DNA-binding or immunoglobulin assays. On the other hand, activated cells from either inactive SLE patients, normal controls, or patient controls were able to significantly suppress ^{3}H-DNA binding to culture supernates and both cytoplasmic Ig and IgG in culture supernates. The only parameter that was not suppressed by the latter two cell sources was ^{3}H-DNA binding to cells. In all the experiments, cells that were not activated with Con A failed to induce significant suppression (Table II). The only exception was nonactivated normal cells which were capable of inducing suppression ($P < 0.05$) with respect to ^{3}H-DNA binding to culture supernates and Ig in supernates.

Con A-activated normal cells cocultured with normal cells in 21 experiments gave values of 4±3 cytoplasmic Ig positive cells and 54±34 ng/ml IgG in supernates. These values were significantly different ($P < 0.001$) from values of cocultures of normal cells incubated with non-Con A-activated normal cells (not shown in Table II). There was no difference between autologous and allogeneic normal cell combinations. No attempt was made to test for ^{3}H-DNA binding to culture supernates or to cells in the co-cultures from normals because of the extremely low counts obtained (4).

Cell type responsible for suppression. As can be seen from Table III in the seven experiments performed, T cells from normal donors, which contained 94±4% T cells, 2±2% B cells, and 2±2% monocytes, were responsible for suppression of the various in vitro parameters of active SLE cells. The B-cell fraction, which contained 91±4% B cells, 11±6% monocytes, and <1% T cells failed to induce suppression.

In Table IV, it is shown that B- and T-cell fractions of mononuclear cells from active SLE donors failed to suppress normal cells.

Target for suppression. As can be seen from Table V, monocyte depletion of cells from the active SLE pa-

TABLE II

Effects of Con A Activation of SLE or Normal Cells on the Various In Vitro Parameters of Active SLE Cells

Cell source	No. studied	Con A activation	Parameters tested* of active SLE cell cultures: ^{3}H-DNA binding to: Culture supernates	^{3}H-DNA binding to: Cells‡	Immunoglobulin: Cytoplasmic§	Immunoglobulin: Supernates‖
			% binding	*no. positive/10^5*	*% positive*	*ng/ml*
Active SLE	14	−	34±7	29±20	22±6	728±125
(autologous)	14	+	39±4	31±14	24±9	591±79
Active SLE	7	−	45±13	21±7	27±11	620±141
(allogeneic)	7	+	29±11	27±14	21±9	720±97
Inactive SLE	7	−	27±9	25±17	20±5	492±137
(allogeneic)	7	+	7±5¶	27±14	4±4¶	98±14¶
Normal	21	−	17±5**	21±10	25±9	409±27**
(allogeneic)	21	+	6±3¶	24±11	5±2¶	<20¶
Controls,	6	−	41±14	19±12	19±7	812±214
patients‡‡	6	+	10±7¶	17±15	5±5¶	55±27¶

* Mean±SD values are shown. In all cases the values of Con A-treated or non-Con A-treated activated cells used to modulate the active SLE cells were subtracted from the values of the co-culture.

‡ Tested by radioautography.

§ Tested by immunofluorescent technique with fluoresceinated anti-human polyvalent antisera.

‖ Tested by immunofluor kits.

¶ $P < 0.001$ when compared to cultures in which no Con A-activated cells were added.

** $P < 0.05$ when compared to cultures of active SLE cells to which no Con A was added (lines 1 and 3).

‡‡ Three patients with polyarteritis nodosa and three with bronchial asthma on a mean prednisone dose of 37.5 and 12.5 mg/day, respectively.

TABLE III

Effects of Con A Activation of Normal Mononuclear Cell Populations on the In Vitro Parameters of Active SLE Cells

Cell type (normal donors)	Con A activation	Parameters tested* of active SLE cell cultures			
		^{3}H-DNA binding to		Immunoglobulin	
		Supernates	Cells*	Supernates*	Cytoplasmic*
		% binding	*no. + ☿/10^5*	*ng/culture*	*% + ☿*
Unfractionated	−	21±7	14±11	520±70	17±7
	+	4±3‡	19±7	<20‡	3±3‡
T	−	11±4	24±9	315±105	14±3
	+	2±1‡	22±5	<20‡	4±2‡
B	−	24±9	17±9	490±110	19±3
	+	23±5	21±14	420±80	18±5

* See footnotes to Table II. Results shown are means±SD of seven experiments.
‡ $P < 0.01$ when compared to results of co-cultures with same cell type that was not activated with Con A.

tients by either iron phagocytosis or plastic adherence methods eliminated the suppressor effects of the Con A-activated normal cells (Table V, lines 4 and 6). Cells from active SLE patients that were not depleted of monocytes could be suppressed ($P < 0.001$) by the Con A-activated normal cells (Table V, lines 3 and 5). Reconstitution of monocyte-depleted cultures by 4.5 × 10^4 plastic adherent autologous cells resulted in suppression of the active SLE cells when co-cultured with the Con A-activated normal cells. The number of adherent cells used was that recovered from 1 × 10^6 plated mononuclear cells. No attempt was made to use different doses of the adherent cells in these experiments.

Effects of SIRS. The optimum volume of SIRS capable of inducing maximal suppression was found to be 0.5 ml. A dose of 0.1 ml was ineffective and 1.0 ml was as effective as 0.5 ml.

As can be seen in Table VI, 0.5 ml SIRS of Con A-activated cells from either inactive SLE or normal donors was capable of inducing significant suppression ($P < 0.05$) of active SLE cutures. The only exception was the inability of SIRS from the same sources to suppress ^{3}H-DNA binding to cells. SIRS obtained from active SLE or control supernates, obtained from cells not activated with Con A, failed to suppress any of the four parameters tested.

SIRS of Con A-activated normal cells added to normal cultures in 16 experiments gave values of 6±5 cytoplasmic Ig positive cells and 97±27 ng/ml IgG in supernates (not shown in Table VI). These values were significantly different ($P < 0.01$) from values

TABLE IV

Effects of Con A Activation of Active SLE Mononuclear Cell Populations on the In Vitro Parameters of Normal Cells

Cell type (active SLE donors)	Con A activation	Parameters tested* of normal cell cultures			
		^{3}H-DNA binding to		Immunoglobulin	
		Supernates	Cells*	Supernates*	Cytoplasmic*
		% binding	*no. + ☿/10^5*	*ng/culture*	*% + ☿*
Unfractionated	−	0	1±1	370±105	12±4
	+	0	2±1	410±120	15±5
T	−	0	1±1	425±75	11±3
	+	0	1±1	395±50	14±5
B	−	0	0	470±60	12±2
	+	0	1±1	405±75	11±3

* See footnotes to Table II. Results shown are means±SD of seven experiments.

TABLE V

Effects of Co-culturing Con A-Activated Normal Mononuclear Cells on Monocyte-Depleted Active SLE Cells

Con A activation (normal donors)	Monocyte depletion (active SLE donors)	Supernates of active SLE cell cultures tested for	
		^{3}H-DNA binding	Immunoglobulin
		*% binding**	*ng/culture**
−	No	19±4	490±105
−	Yes‡	22±7	555±90
+	No	5±4§	<20§
+	Yes‡	25±5	615±205
+	No	3±3§	<20§
+	Yes‖	14±3	710±120
+	Reconstitution¶	5±4**	<20§

* See footnotes to Table II. Mean±SD of seven experiments is shown.

‡ Depletion by iron phagocytosis and magnet.

§ $P < 0.001$ when compared to values of monocyte-depleted co-cultures.

‖ Depletion by adherence to plastic surface.

¶ Depletion by adherence to plastic surface followed by addition of 4.5×10^4 autologous monocytes (Methods). Four experiments were performed.

** $P < 0.05$ when compared to monocyte-depleted co-cultures done by the same method.

of cultures of normal cells incubated with the control supernates which were raised from normal cells in the absence of Con A. That the source of SIRS is from autologous or allogeneic normal cells did not change the suppressive capacity. No attempt was made to test for ^{3}H-DNA binding to culture supernates or to cells of normals' cultures because of the extreme low counts obtained (4).

In Table VII, it can be seen that monocyte depletion from cultures of active SLE donors, eliminated the suppressive potential of SIRS obtained from Con A-activated normal cells (Table VII, line 4). Cells from active SLE patients that were not depleted of monocytes could be suppressed ($P < 0.05$) by the Con A SIRS (Table VII, line 3). Reconstitution of monocyte-depleted cultures by 4.5×10^4 plastic adherent autologous cells resulted in suppression of the active SLE cells when co-cultured with SIRS harvested from Con A-activated normal cells (Table VII, line 7).

DISCUSSION

The pathogenesis of the autoimmune abnormalities in human SLE and the NZB/W mouse model has been clarified recently by the discovery of T-cell suppressor dysfunction (4, 5, 17, 18). The expansion and(or) activation of the autoreactive clones (19, 20) may be a result of abnormal immunoregulation secondary to the suppressor cell abnormality (2, 3, 21). Normal healthy people have few autoreactive clones which are kept under strict control by active normal suppressor cells which prevent their autodestructive capability.

The observation from several laboratories that Con A can activate suppressor cells (7, 8) which secrete suppressor factors has opened a new tool for the possible correction of the T-cell suppressor dysfunction in autoimmunity.

In this report we have shown that immunoglobulin and anti-DNA secretion by active SLE cells can be inhibited by Con A-activated cells or SIRS obtained

TABLE VI

Effects of SIRS from SLE or Normal Cells on the Various In Vitro Parameters of Active SLE Cells

SIRS source	No. studied	Type of SIRS	Parameters tested* of active SLE cell cultures			
			^{3}H-DNA binding to		Immunoglobulin	
			Culture supernates	Cells‡	Cytoplasmic§	Supernates‖
			% binding	*no. positive/10^5*	*% positive*	*ng/ml*
Active SLE	9	Control	31±11	21±7	17±3	679±109
		Con A	27±7	25±6	21±7	561±94
Inactive SLE	5	Control	29±5	29±11	19±7	642±59
		Con A	13±3¶	20±5	6±3¶	204±23¶
Normal	16	Control	25±9	32±9	23±9	573±94
		Con A	11±3¶	27±5	7±7¶	89±39¶

* Means±SD values are shown.

‡ Tested by radioautography.

§ Tested by immunofluorescent technique with fluoresceinated anti-human polyvalent antisera.

‖ Tested by immunofluor kits.

¶ $P < 0.05$ when compared to cultures in which control SIRS were added.

TABLE VII
Effects of SIRS from Normal Cells on Monocyte-Depleted Active SLE Cells

Type of SIRS (from normal donors)	Monocyte depletion (active SLE donors)	Supernates of active SLE cultures tested for	
		^{3}H-DNA binding	Immunoglobulin
		*% binding**	*ng/culture**
Control‡	No	30±12	529±127
Control	Yes§	27±7	605±69
Con A‡	No	9±4‖	212±44‖
Con A	Yes§	22±5	553±97
Con A	No	12±3‖	147±19‖
Con A	Yes¶	37±11	712±64
Con A	Reconstitution**	8±4‖	164±62‖

* Mean±SD of seven different experiments.
‡ Supernates of 48 h normal cell cultures to which no Con A (control) or Con A (Con A) was added.
§ Depletion by iron phagocytosis and magnet.
‖ $P < 0.05$ when compared to values of cultures depleted of monocytes.
¶ Depletion by adherence to plastic surface.
** Depletion by adherence to plastic surface followed by addition of 4.5×10^4 autologous adherent cells (Methods). Four experiments were performed.

from normal cells, from SLE cells of inactive patients, or from patients with polyarteritis nodosa or asthma who were on oral prednisone in doses equivalent to those which the active SLE patients used. Cells from active SLE patients cannot generate suppressor cells or SIRS, although they can respond to suppressor signals from the normal cells. Upon fractionation of cells into T-, B-, and monocyte-depleted populations, we have clearly shown that T cells are responsible for the suppressor effects or for the generation of SIRS. Depletion of cells capable of phagocytosing iron and sticking to a magnet or of plastic adherent cells from active SLE patients has indicated that the cells which respond to the suppressor signals were probably monocytes. It seems, therefore, that both T cells and monocytes are required for the successful suppression of the various in vitro parameters tested. The former generates soluble suppressor factors and the latter responds to those suppressor signals. The mechanism by which monocytes suppress anti-DNA antibody and immunoglobulin production in vitro still remains to be seen.

It seems that the suppressor dysfunction in active SLE is reversible. We have shown that cells from the same SLE patients can generate SIRS when their disease becomes inactive. This would indicate that the suppressor dysfunction in SLE is not a permanent progressive state as was observed in the NZB mice (3, 5, 18). This would make it tempting to prolong the remission of human SLE by correcting suppressor cell dysfunction by the prophylactic and prolonged administration of SIRS. The number of DNA-binding cells during the short period of incubation of those cells with the SIRS did not decrease and is unexplained. It is possible that long-term in vivo treatment by SIRS might successfully decrease the numbers of DNA-binding cells by a longer contact with active suppressor cells.

The cause(s) of suppressor cell dysfunction in SLE is unknown. Viral infections (22, 23), anti-T-cell antibody (24, 25) or antimonocyte antibody (26), could be possible mechanisms for qualitatively altering T-cell function. It is also unclear whether the T-cell suppressor dysfunction precedes or follows the autoimmune B-cell hyperactive state. In the NZB/W animal model, suppressor dysfunction seems to precede the autoimmune state (5, 18).

Further clarification of the mechanism by which suppressor cells or SIRS regulate autoreactive clones may lead to specific immunoregulation as a tool in the management of active SLE. Induction of suppressor T cells in SLE by thymosin and cultured thymic epithelium has recently been reported (27). These therapeutic modalities are nonspecific and, therefore, the possibility of suppressing normal clones that may be important in host defense mechanisms against infections (28) and tumors (29, 30) would have to be carefully studied before in vivo application is considered.

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