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Studies of Immune Functions of Patients with Systemic Lupus Erythematosus: *COMPLEMENT-DEPENDENT IMMUNOGLOBULIN M ANTI-THYMUS-DERIVED CELL ANTIBODIES PREFERENTIALLY INACTIVATE SUPPRESSOR CELLS*

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Patients with systemic lupus erythematosus (SLE) produce excessive amounts of autoantibodies. It has also been demonstrated in several systems that such patients have a relative loss of suppressor thymus-derived (T) cells that inhibit the immune response. This loss of suppressor cells has been suggested as one of the causes of the excessive production of antibodies in patients with SLE.

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ABSTRACT Patients with systemic lupus erythematosus (SLE) produce excessive amounts of autoantibodies. It has also been demonstrated in several systems that such patients have a relative loss of suppressor thymus-derived (T) cells that inhibit the immune response. This loss of suppressor cells has been suggested as one of the causes of the excessive production of antibodies in patients with SLE.

In the present report we have tested the hypothesis that anti-T-cell antibodies found in the plasma of some patients with SLE preferentially kill suppressor cells. T cells from normal individuals can be activated by concanavalin A to develop suppressor cell activity. We therefore cultured normal T cells together with concanavalin A in the presence of plasma or plasma fractions from patients with SLE. We found that plasma from patients with active SLE, in which anti-T-cell antibodies were present, inhibited the development of suppressor activity in such cultures. In contrast, plasma from other active patients and patients with inactive SLE, in which no anti-T-cell antibodies could be detected, failed to block the development of such suppressor activity. Absorption of the plasma that contained anti-T-cell antibodies with T cell, but not non-T cells, could eliminate the suppressor-inhibiting activity of the SLE plasma that contained anti-T-cell antibodies. The immunoglobulin (Ig)M, but not the IgG, fraction of the plasma was shown to possess the inhibiting property and complement was found to be necessary for the effect of such anti-T-cell antibodies. We also demonstrated that exposure of normal T cells to such anti-T-cell antibodies and complement did not affect another population of T cells that could proliferate in response to mitogens.

Thus, certain patients with SLE have in their plasma an antibody of the IgM class that can selectively eliminate a population of T cells capable of developing suppressor function. The loss of suppressor T cells in patients with SLE may be the result of the effects of such antibody activity in vivo.

INTRODUCTION

Patients with active systemic lupus erythematosus (SLE)[1] manifest a variety of immunological abnormalities of both thymus-derived (T) and bone marrow-derived (B) cells. These include a reduction in the number of T cells in their peripheral blood (1–3), and impairment of several T-cell functions (4–7). In addition, active SLE is associated with excessive B-cell activity (8–12). This B-cell hyperactivity is generalized and is not limited to production of autoantibodies. Nevertheless, increased production of autoantibodies is one of the predominant feature of patients with SLE. These antibodies, especially in the form of immune complexes, are thought to be related to many of the disease manifestations in these patients (13, 14).

Recently there has been great interest in the regula-

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[1] *Abbreviations used in this paper*: Con A, concanavalin A; [^{3}H]thymidine, [*methyl*-^{3}H]thymidine; MLR, mixed-lymphocyte reactions; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SLE, systemic lupus erythematosus; SRBC, sheep erythrocytes.

tion of the immune response, particularly with regard to suppressor cell activity (15). A number of different suppressor T-cell systems, some specific and others nonspecific, have now been described (16–20). We have been investigating concanavalin A (Con A)-induced suppressor T-cell functions in normal individuals and in patients with SLE. In our previous studies we have demonstrated that this Con A-induced suppressor T-cell function is impaired in most patients with active SLE (21). In addition, we found that this defect resided in the generation of suppressor cells, and not in the response to suppressor cell signals. The reason for this defect in the generation of suppressor T cells has remained elusive. Some investigators have proposed that this defect, which does not vary with disease activity, is corrected by thymic hormones and is a primary genetic defect (22). Another hypothesis is that anti-T-cell antibodies could be responsible for a loss of suppressor cells (23) as has been found in New Zealand mice with a lupus-like syndrome (24).

Patients with SLE are known to spontaneously produce antibodies to lymphocyte membrane antigens (23, 25–40). These antibodies have been chiefly identified by their ability to bind to and(or) kill lymphocytes; fewer studies have attempted to test the functional capabilities of lymphocytes after interaction with these antibodies. In particular, it is not known whether or not these SLE anti-T-cell antibodies are largely responsible for the functional lymphocyte defects described in patients with SLE.

In the present study we have investigated whether plasma from SLE patients could alter immune regulatory functions. We found that such plasma inhibited the development of Con A-induced suppressor T cells obtained from normal individuals. Furthermore, the subclass of antibodies involved and the requirement of complement was determined. These studies provide evidence for a role of anti-T-cell antibodies in the development of one of the immune abnormalities of active SLE patients.

METHODS

Overall experimental design. To approach the question as to why patients with SLE have a defect in the development of suppressor T cells (21, 22, 41, 42), we have examined the effect of SLE plasma on the generation of suppressor cells. We have taken advantage of the observations that human peripheral blood cells can be activated by Con A to become cells that suppress the proliferative responses of responder cells autologous with the Con A-activated cells (16). Such Con A-induced suppressor cells were shown to be T cells (17).

We therefore cultured T cells obtained from normal individuals in the presence of Con A with unfractionated whole plasma, or plasma fractions from SLE patients, to determine whether this treatment could block the development of suppressor cells. As control for these experiments with SLE plasma, plasma obtained from normal individuals was also used. T cells from normal individuals treated with normal or SLE plasma plus complement were also examined for responsiveness to stimulation with mitogens and allogeneic cells to determine whether the SLE plasma could affect other T-cell functions.

Mononuclear cell isolation. Peripheral blood from healthy human donors (obtained from the Blood Bank Department, Clinical Center, National Institutes of Health, Bethesda, Md.) was diluted with heparinized saline (10 U/ml blood). Heparinized blood was diluted with an equal volume of Hanks' balanced salt solution. 40 ml of the diluted blood was layered over 10 ml of Ficoll-Hypaque solution (Ficoll-Paque, Pharmacia Fine Chemicals, Piscataway, N. J.) and centrifuged at 4°C for 30 min at 400 *g*. The mononuclear cells were then removed from the interface and washed three times in Hanks' balanced salt solution.

Purification of T cells, non-T cells, and monocytes. The T cells, non-T cells, and monocytes were purified as described (43). Neuraminidase-treated sheep erythrocytes (SRBC) and the washed mononuclear cells were mixed in a ratio of 40:1 in Hanks' balanced salt solution supplemented by 25% absorbed fetal bovine serum (Microbiological Associates, Walkersville, Md.). The SRBC-mononuclear cell suspension was incubated at 37°C for 15 min, spun at 200 *g* for 10 min, and incubated on ice for 1 h. 20 ml of the suspension was layered over 10 ml Ficoll-Hypaque, centrifuged at 400 *g* for 30 min at 4°C, and rosetted cells in the pellet fraction and unrosetted cells at the interface were collected separately. The pelleted population was rerosetted with SRBC exactly as described above and recentrifuged over Ficoll-Hypaque. The pelleted cells from the final Ficoll-Hypaque gradients were resuspended in 0.83% ammonium chloride to lyse the SRBC. These cells were >95% pure T cells as judged by rerosetting and are designated T cells. The unrosetted population obtained from the first cycle of the SRBC-rosette procedure was also subjected to further purification by rerosette formation with SRBC and subsequent density centrifugation. The interface cells obtained from the second cycle of the purification procedure were resuspended in RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% heat-inactivated fetal bovine serum, and incubated on Petri dishes at 37°C for 2 h in 5% CO_2. Adherent and nonadherent cells were harvested separately. We refer to the nonadherent cell population as non-T cells and the adherent cell population as "monocytes". The percentage of T-cell contamination in the non-T cell preparations was <1%. Monocyte preparations consisted of 95% cells that were identified as monocytes after Giemsa staining.

Patients and source of plasma. Adult patients satisfying the diagnostic criteria of the American Rheumatism Association for SLE were admitted to the Arthritis Branch of National Institute of Arthritis, Metabolism, and Digestive Diseases at the Clinical Center, National Institutes of Health. Plasma was obtained from patients before treatment and was also obtained after treatment as indicated (Table V). At the time of blood drawing, patients with active disease had not yet received any treatment. Inactive patients had previously been treated with corticosteroids and occasionally with azathioprine, but were not receiving such treatment at the time of study. Clinical activity was assessed at the time of blood drawing by two physicians on the basis of signs and symptoms (active rash, serositis, arthritis, active central nervous system disease, and active renal disease). Patients lacking these symptoms or detectable signs of activity were categorized as inactive. The active patients in this study had at least three of the above criteria of activity. In addition, they all had high titers of antibodies to native DNA. The antibodies to purified human T cells were measured by indirect immunofluorescence with flow microfluorometry as

described (44). 9 of 13 patients with active SLE had anti-T-cell antibodies, whereas none of the inactive SLE patients had antibodies to T cells. All the SLE plasma used had been fresh frozen and had not been previously thawed. Normal fresh frozen plasma was obtained from healthy adults. All plasma was centrifuged at 105,000 *g* for 2 h at 4°C to remove aggregated materials before use.

Adsorption of plasma with normal T cells or non-T cells. T cells or non-T cells used for adsorption were prepared from normal individuals as described above. Plasma either from SLE patients or a normal individual was incubated with 2.5–3.0 × 10^8 packed T cells or non-T cells/ml plasma at 4°C overnight. Thereafter the cells were removed by centrifugation at 105,000 *g* for 2 h at 4°C and the supernatant plasma collected.

Removal of immunoglobulin (Ig)M or IgG component from plasma by precipitation with antiserum for human IgM or IgG. The IgG fraction of rabbit anti-human IgM and the IgG fraction of rabbit anti-human IgG were purchased from N. L. Cappel Laboratories Inc., Cochranville, Pa. These antisera against IgM and IgG were dissolved in sterile distilled water at concentrations of 60 and 400 mg/ml, respectively. 20 μl of each antiserum was added to separate 1-ml aliquots of plasma from a patient. After incubation overnight at 4°C, the precipitate was removed by centrifugation at 105,000 *g* for 2 h at 4°C. This precipitation procedure was repeated again, and the supernates were collected. These supernates were shown to be depleted of the respective immunoglobulins by radial immunodiffusion of 20-fold concentrated samples.

Preparation of IgG and IgM fractions of plasma by Sephadex G-200 column chromatography. Plasma was precipitated with 50% ammonium sulfate, dialyzed against buffer, and applied to a 1.5-meter-long Sephadex G-200 (Pharmacia Fine Chemicals) column. Individual fractions were collected and the optimal density at 280 nm of each fraction measured in a spectrophotometer. Marker proteins were run to confirm the approximate size of molecules obtained from the resulting peaks. A good separation of IgM and IgG peaks was observed. The purity of each fraction was confirmed by radial immunodiffusion of 20-fold concentrated samples. To further check their purity, the peaks were separately rechromatographed; no IgG was found in the IgM fraction and no IgM was found in the IgG fraction. The IgM and IgG peaks were separately pooled and concentrated. Individual fractions were dialyzed overnight against phosphate-buffered saline, pH 7.2.

Generation of suppressor T cells by activation with Con A. Because previous studies in our laboratory have shown that Con A-activated cells able to suppress proliferative responses reside only in the T-cell population, and not in the non-T cell population (17), purified T cells from normals were used in a culture for the generation of suppressor cells by Con A activation. Thus, 4 × 10^6 T cells were incubated in 4 ml culture medium, RPMI 1640 supplemented with 100 U penicillin/ml, 100 μg streptomycin/ml, 2 mM L-glutamine, 25 mM Hepes buffer, and 10% various test plasma (see below) in 17 × 100 mm plastic tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.); these mixtures were incubated at 4°C for 1 h and were then kept at room temperature for another 3 h. Thereafter, 3 × 10^5 mitomycin-treated (Sigma Chemical Co., St. Louis, Mo.) monocytes were added and incubated at 37°C in a 5% CO_2-95% air humidified environment with 40 μg Con A (Con A-activated T cells) (Pharmacia Fine Chemicals) or without Con A (nonactivated control T cells). 60 h later, the cells were harvested, washed four times, treated with mitomycin, and then tested for their suppressor activity in the assay culture system (see below).

When the effect of unfractionated whole plasma from patients with SLE on generation of suppressor T cells was investigated, the culture medium used for Con A activation was composed of RPMI 1640 and nonheated plasma at a final concentration of 10%. It should be noted here that adequate amounts of complement were present, because experiments shown in Tables I and II were repeated with the addition of 5% fresh frozen normal human plasma as a source of complement with essentially the same results. In addition, use of normal serum instead of plasma as a source of complement gave similar results.

The preparation procedures of plasma absorbed with either T cells or non-T cells and of plasma from which IgM or IgG component had been removed by precipitation could have caused decrease in complement activity of the plasma. Therefore, when such plasma was used, normal T cells were incubated with or without Con A in RPMI 1640 that contained 5% of the plasma to be studied plus 5% fresh plasma autologous with T cells as complement source. 5% plasma was used in the above experiment because preliminary study revealed that there was no difference between inhibitory effect on generation of suppressor cells by 5 or 10% SLE plasma. To test the effect of IgG or IgM fraction of plasma prepared by Sephadex G-200 column chromatography and requirement of complement, the fraction was added at a concentration of 50 μg/ml culture medium, RPMI 1640, and 10% fresh autologous plasma (complement source) or 10% heat-inactivated (56°C 60 min) autologous plasma (removal of complement activity), in the Con A-activated culture. It should be noted that in some experiments with the IgG and IgM fractions, use of fresh frozen, normal serum instead of autologous plasma as a complement source gave identical results.

Assay for suppressor activity of Con A-activated T cells in the assay culture system. T cells that were precultured with Con A in the presence of the various test plasma or plasma fractions were then tested for their suppressive ability of proliferative responses in the assay culture system. Responder cells to be used in the assay cultures were obtained 3 d later from the same normal individual who originally provided the suppressor cells. Purified T cells and non-T cells that were used as responder cells were prepared exactly as described above. In the assay cultures, 1 × 10^5 mitomycin-treated, Con A-activated or nonactivated control T cells were added to 1 × 10^5 responder cells and stimulated with either allogeneic cells or pokeweed mitogen (PWM) (Grand Island Biological Co.). To fully develop the suppressor activity by Con A-activated cells, mitomycin-treated monocytes (5,000/culture) that had been obtained from the second bleeding were also added to the assay culture system (45). In the assay cultures, RPMI 1640 plus 10% heat-inactivated fetal bovine serum was used as the assay culture medium. Where T cells were used as responder cells in the assay culture, they were stimulated with 1 × 10^5 mitomycin-treated allogeneic stimulating cells. When non-T cells were used as responder cells, PWM (2 μg/ml) was the stimulant in the culture. Because the non-T cell response to PWM is clearly T-cell dependent (46), freshly prepared autologous T cells that had been treated with mitomycin were also added (5 × 10^4 per culture). All cultures were performed in triplicate in 0.2 ml in microtiter plates (Cooke Engineering Co., Alexandria, Va.) and incubated for 144 h at 37°C in a 5% CO_2, 95% air humidified environment. At 20 h before the termination of the incubation period, 1 μCi of [*methyl*-^{3}H]thymidine ([^{3}H]thimidine) (5 Ci/mmol; Amersham

Corp., Arlington Heights, Ill.) was added to each culture well. At the end of the incubation period, the cells were processed on a microharvester and incorporation of [^{3}H]-thymidine was measured by liquid scintillation counting. The degree of suppression was calculated with the following formula: percentage of suppression = (1 − [mean counts per minute of stimulated cultures containing Con A-activated T cells − mean counts per minute of unstimulated cultures containing Con A-activated T cells]/[mean counts per minute of stimulated cultures containing non-activated T cells − mean counts per minute of unstimulated cultures containing non-activated T cells]) × 100.

Evaluation of other T-cell functions after treatment with plasma and complement. To determine whether or not treatment with SLE anti-T-cell antibodies indiscriminately killed T cells and thus removed T-cell functions, T cells were preincubated with a large quantity of previously studied plasma for a long period of time to achieve maximal killing. We then tested the resulting T cells for their residual ability to respond to T-cell mitogens and allogeneic cells. Thus, 5×10^6 normal T cells were mixed with 1 ml plasma from either four active SLE patients with anti-T-cell antibodies or two normal individuals at 4°C overnight. These T cells were then incubated with 1 ml fresh normal human serum as a complement source at room temperature for another 5 h. Thereafter, these cells were washed three times and then tested for their ability to proliferate in response to phytohemagglutinin (PHA; The Wellcome Research Laboratories, Beckenham, England), Con A, and allogeneic cells as previously described (17, 21). Briefly, cell counts and viabilities were first determined and cell numbers were adjusted appropriately. All cultures were established in 0.2 ml of culture medium, RPMI 1640 and 10% heat-inactivated fetal bovine serum, that contained 1×10^5 viable T cells resulting from the prior treatment with plasma plus complement, and stimulated with optimal concentrations of stimulants; PHA (1 μg/ml), Con A (10 μg/ml), or 1×10^5 mitomycin-treated allogeneic cells. To obtain maximum responses, mitomycin-treated monocytes (5,000/culture), autologous with the responding T cells were also added to the cultures. The responses to PHA and Con A generated during the incubation time of 72 h, and those to allogeneic cells generated during the incubation time of 144 h were measured in a [^{3}H]thymidine incorporation assay as described above.

RESULTS

Inhibitory effect of SLE plasma on generation of Con A-induced suppressor T cells. Con A activation of purified T cells from normal donors leads to the generation of cells capable of suppressing the mixed-lymphocyte reactions (MLR), PWM-driven proliferation, and other immune functions. The T cells of most patients with SLE are defective with regard to generation of these Con A-induced suppressor cells. To determine whether or not this defect in T cells from patients with SLE is related to a plasma factor, we mixed SLE plasma with normal T cells during the period of Con A activation, and tested the subsequent suppressive capacity of the activated cells. Such an experiment is shown in Table I. Plasma from a single patient with active renal and extrarenal disease containing a high titer of anti-T-cell antibodies (determined by indirect immunofluorescence) almost completely prevented the generation of cells capable of suppressing the MLR and PWM responses. In contrast, the control plasma from a normal individual did not prevent the generation of suppressor cells. In this experiment, the SLE plasma-treated cell population contained only 69% of control numbers of viable cells after the incubation period. However, the number of viable cells added to each assay culture to be tested for their suppressor activity was adjusted so that each contained the same standard number of viable cells.

To determine whether or not the above experiment was unique to plasma from this single patient, plasma from additional active and inactive SLE patients was studied and the results summarized in Table II. Heat-inactivated fetal bovine serum served as a control in all experiments. Plasma from normal individuals did not affect the generation of suppressor cells. The degree of suppression observed after treatment of the cells with plasma not containing anti-T-cell antibodies from inactive SLE patients did not differ significantly from that observed with plasma from the normal individuals. When plasma from active SLE patients that did not contain anti-T-cell antibodies was used, the results were the same; no interference with the development of suppressor cells. In contrast, incubation of cells with plasma from active SLE patients that contained anti-T-cell antibodies markedly impaired the generation of suppressor cells for both the MLR ($P < 0.005$) and PWM ($P < 0.02$) responses as compared with the other groups. These results indicate that only plasma that contained anti-T-cell antibodies could block the development of suppressor cells.

Specificity of SLE plasma factor. The possible specificity of the factor present in active SLE plasma responsible for the elimination of suppressor cells was studied by absorption of plasma that contained anti-T-cell antibodies with either normal T cells or non-T cells (Fig. 1). Absorption with T cells almost completely removed the activity of the SLE plasma. In contrast, absorption with non-T cells did not remove the activity; in three instances, the blocking activity of the plasma was actually further enhanced by absorption with non-T cells (Fig. 1). As expected, there was no effect of normal whole plasma on blocking of suppressor function whether or not it was absorbed.

In addition, to determine whether the presence of aggregated immunoglobulin had any effect, human 7S gamma globulin fraction II was heat aggregated at 63°C for 15 min as described (43). 1 mg/ml of this heat-aggregated IgG was added at the initiation of the Con A-treated T-cell cultures. In two separate experiments, this procedure did not inhibit the generation of suppressor cells (data not shown).

Effects of IgG and IgM fractions from active SLE

TABLE I

*Effect of Plasma from an Active SLE Patient with Anti-T-Cell Antibodies on the Generation of Con A-Induced Suppressor T Cells; a Representative Experiment**

Con A-activated culture		Suppressor activity in assay culture			
Plasma source	Con A	MLR‡		PWM§	
		Δcpm±SE	% suppression	Δcpm±SE	% suppression
Normal	−	18,989±343		21,439±1,580	
	+	6,304±822	66.8	10,430±840	51.4
SLE	−	18,184±635		19,039±1,581	
	+	15,184±352	16.5‖	18,106±874	4.9‖

* Normal T cells were incubated for 60 h with Con A or without Con A in culture medium supplemented with 10% unheated plasma either from a normal individual or from an active SLE patient with anti-T-cell antibodies. These T cells were added to freshly prepared autologous responder cells and then stimulated for 144 h with either allogeneic cells or PWM. Proliferative response was measured by incorporation of thymidine during the last 20 h of culture period, and percentage of suppression was calculated as described in Methods.
‡ T cells autologous with Con A-induced suppressor cells were used as responder cells and allogeneic cells used as stimulator cells in this assay culture.
§ Non-T cells were used as responder cells in the assay culture.
‖ Significantly different from effect of normal plasma, $P < 0.005$.

plasma. Two methods were used to determine whether the effective fraction of SLE plasma that contained anti-T-cell antibodies was an antibody of the IgG or IgM class. First, active SLE plasma was reacted with precipitating antibody specific for either IgM or IgG. The supernatant fractions were then tested for their ability to interfere with the generation of suppressor cells (Table III). Precipitation with antiserum to IgM removed the ability of the plasma to block the generation of suppressor cells. In contrast, precipitation with antiserum to IgG had no effect on the activity of the SLE plasma. Thus the activity seemed to reside in the IgM fraction.

Second, the class of antibodies required was further studied by isolation of the IgG and IgM fractions by column chromatography. Initially, we show the results with fractions of a single SLE plasma that contained anti-T-cell antibodies (Table IV). In this study, the requirement of complement was also studied. Autologous fresh plasma was used as a source of complement. The control for this was the use of heat-inactivated (56°C for 60 min) normal autologous plasma. Both the heated and unheated autologous plasma was centrifuged at 105,000 *g* for 2 h to remove aggregated gamma globulin and other aggregated materials. As in the preprecipitation study (Table III), it was found that the inhibitory activity of the active SLE antibody for generation of suppressor cells was in the IgM fraction, but not in the IgG fraction (Table IV). Furthermore, complement was necessary for the effect of the antibody (Table IV). Fig. 2 gives a summary of such experiments with the plasma of six patients with active SLE. Four of the plasma contained anti-T-cell antibodies; the other two plasma were devoid of detectable anti-T-cell antibodies. IgM, but not IgG, column fraction of plasma that contained anti-T-cell antibodies reduced or abolished suppression. This effect was dependent upon complement. The IgG and IgM fractions obtained from the other two plasma without anti-T-cell antibodies and from one normal individual were also examined. There was no significant effect of these plasma fractions on the development of suppressor T cells (Fig. 2).

Effect of plasma from a patient before and after treatment. Plasma was first obtained from a SLE patient with active central nervous system and renal disease; at that time anti-T-cell antibodies were found in the plasma. This patient was treated and 5 yr later, when clinically asymptomatic, plasma was again obtained. Both plasma were frozen and subsequently studied (Table V). In both studies control frozen plasma from a normal person was also investigated simultaneously. T cells treated with this normal plasma demonstrated generation of suppressor cells. However, plasma from the patient, when active, completely inhibited the generation of suppressor cells for both

TABLE II

*Effect of Plasma from Patients with Active or Inactive SLE on the Generation of Con A-Induced Suppressor T Cells**

Plasma source in Con A-activated culture		Suppressor activity in assay culture	
		MLR‡	PWM§
		% suppression±SE	
Fetal bovine serum‖		60.6±2.9	51.7±4.4
Plasma from normal individuals	(11)¶	56.8±2.7	54.8±3.9
Plasma from active SLE patients with anti-T-cell antibodies	(8)¶	14.1±4.6**	1.4±10.1**
Plasma from active SLE patients without anti-T-cell antibodies	(4)¶	56.1±9.3	49.5±6.8
Plasma from inactive SLE patients (none had anti-T-cell antibodies)	(5)¶	52.5±11.9	64.4±1.5

* Normal T cells were incubated with Con A or without Con A in culture medium that contained 10% unheated plasma obtained from normal individuals, active SLE patients, or inactive SLE patients. These cells were introduced into freshly prepared autologous responder cells in the assay culture which were stimulated with either allogeneic cells or PWM.

‡ Same as in Table I.

§ Same as in Table I.

‖ Heat-inactivated fetal bovine serum was used as a control in all experiments.

¶ The number of individual plasma is shown in parenthesis. The results of one study with each plasma is shown.

** Significantly different from effect of plasma obtained from the other groups ($P < 0.02$).

MLR- and PWM-induced proliferation. Plasma obtained during the clinically inactive period interfered with the generation of suppressor cells for the MLR, but not for the proliferative response to PWM.

Functional capacity of T cells remaining after treatment with anti-T-cell antibodies and complement. To determine whether or not the SLE antibodies eliminated all functional T cells, or only particular subsets of T cells, additional experiments were performed. Normal T cells were incubated with SLE plasma that contained anti-T-cell antibodies followed by fresh normal serum as a source of complement, and tested for ability to respond to stimulation with PHA, Con A, or allogeneic cells. All these plasma had been shown in the present study to be capable of blocking the generation of suppressor cells. Such SLE plasma generally reduced the number of T cells by

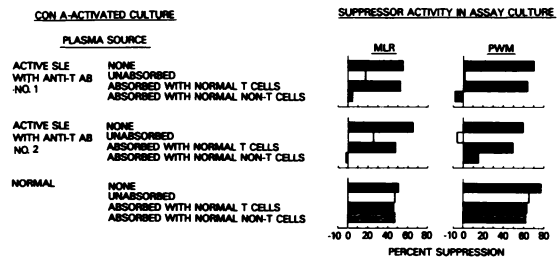

FIGURE 1 Effect of SLE plasma absorbed with normal T cells or non-T cells on the generation of suppressor T cells. Plasma from two active SLE patients with anti-T-cell antibodies and from a normal individual was first absorbed with either normal T cells or non-T cells. The absorbed plasma to be studied was mixed with normal T cells during the culture period of Con A activation. These Con A-activated T cells were then tested for their suppressor activity in the assay culture.

20–30%. In one case, as many as 72.4% of the T cells were killed (Table VI). As shown in Table VI, the T-cell populations that remained after this treatment responded normally to PHA and Con A. In contrast, the response to allogeneic cells was significantly diminished in three out of four cases. These results suggest that SLE plasma could interfere with the functions of some T-cell subsets without affecting the functions of other subsets. In this study, we did not perform dose-response studies for the T-cell mitogens and allogeneic cells. We only employed the concentration of each stimulant at which maximal stimulation of untreated T cells was observed. Therefore, it is possible that under different conditions, greater or lesser responses of treated T cells might be observed.

TABLE III

*Effect of Removal of IgG or IgM Antibody by Precipitation from Plasma from an Active SLE Patient with Anti-T-Cell Antibodies on the Development of Suppressor Cell**

Plasma source added	Antibody used for precipitation	Suppression	
		MLR‡	PWM§
		%	
Fetal bovine serum	—	77.4	75.2
SLE	—	22.3	15.5
SLE	Anti-IgM	71.9	76.6
SLE	Anti-IgG	24.5	29.4

* Normal T cells were incubated in culture medium that contained 5% autologous fresh plasma plus 5% unprecipitated or precipitated plasma from an active SLE patient with anti-T-cell antibodies or fetal bovine serum throughout the period of Con A activation. These T cells were then added to assay cultures that contained freshly prepared autologous responder cells.

‡ Same as in Table I.

§ Same as in Table I.

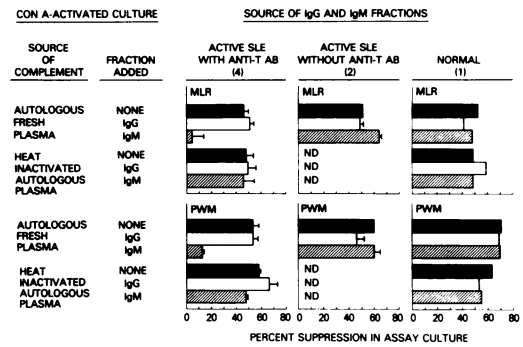

FIGURE 2 Summary of experiments on the effect of IgG and IgM fractions of plasma from active SLE patients and requirement of complement. IgG or IgM fraction of plasma (50 μg/ml) from active SLE patients with or without anti-T-cell antibodies, or from a normal individual was mixed with normal T cells in the presence of culture medium that contained either fresh plasma autologous with T cells (complement source) or heat-inactivated autologous plasma (removal of complement activity) and incubated with Con A. These Con A-activated T cells were then tested for their suppressor activity in the assay culture system. The number of plasma tested is in parenthesis. The mean percentage of suppression ± 1 SE is shown. ND, not done.

DISCUSSION

Patients with SLE have been found to have reduced numbers of recirculation lymphocytes (1, 47, 48), especially T cells (1–3). Furthermore, a variety of T-cell abnormalities have been recorded in patients with active SLE (4–7, 21, 22, 41, 42, 49). The known antilymphocyte and anti-T-cell antibodies found in patients with SLE, could be responsible for both the numerical and functional abnormalities. The present study lends support to this possibility. We found that plasma from patients with active SLE, in which anti-T-cell antibodies were present, inhibited the development of suppressor activity in the cultures of normal T cells activated by Con A. In contrast, plasma from other active patients and patients with inactive SLE, in which no anti-T-cell antibodies could be detected, failed to block the development of such suppressor activity. Absorption of the plasma that contained anti-T-cell antibodies with T cells, but not non-T cells, could eliminate the suppressor inhibiting activity of the SLE plasma that contained anti-T-cell antibodies. The IgM, but not the IgG, fraction of the plasma was shown to possess the inhibiting property and complement was found to be necessary for the maximal effect of such anti-T-cell antibodies. Finally, it is unlikely that the observed results can be attributed to binding of immune complexes to Fc receptors because the incubation of T cells with Con A in the presence of 1 mg/ml of aggregated gamma globulin failed to interfere with the generation of suppressor cells. A recent preliminary study by Twomey et al. (50) also showed that whole sera from patients with SLE inhibit the development of suppressor function by normal T cells precultured with Con A.

It has been previously shown that both IgG and IgM antibodies directed against T cells are present in the sera of patients with SLE. Antibodies cytotoxic for lymphocytes in SLE sera have been found primarily in the IgM class (25, 27, 51), whereas noncytotoxic antibodies were more likely to be of the IgG class (34, 52). Furthermore, antibodies of the IgG class have been demonstrated to functionally impair certain T-cell responses such as ability to proliferate in response to mitogens and allogeneic cells (5, 32, 34, 53, 54). It is, however, conceivable that previous reports of IgG anti-T-cell antibodies may have also involved binding of IgG-containing immune complexes binding to T cells by way of the Fc receptor on some T cells.

Our finding that IgM, but not IgG, anti-T-cell antibodies could prevent the generation of suppressor cells is one of the few reports of an IgM antibody from patients with SLE capable of interfering with an immunological function of T lymphocytes. The reason that we did not find IgG antibodies which could block the development of suppressor cells in the present study could be explained in several ways: (*a*) The amounts of IgG antibodies in the particular plasma tested may have been lower than the amounts of IgM antibodies, thereby not giving a positive result at the quantities used; (*b*) multivalent binding by the IgM antibodies may provide for a much greater total affinity for T cells than might be possible for IgG antibodies; or (*c*) IgM antibodies may be more efficient than IgG antibodies as regard to cytotoxicity. This last possibility is supported by a previous study demonstrating that the cytotoxic capacity of IgM anti-Thy 1 (theta) antibodies was hundreds of times greater than that of IgG anti-Thy 1 antibodies (55). Furthermore, anti-T-cell cytotoxic antibodies, which occur spontaneously in autoimmune New Zealand mice, have also been found to be largely, if not exclusively, of the IgM class (56, 57).

The ability of certain anti-T-cell antibodies to selectively eliminate a functional subpopulation of lymphocytes is well established in mice. Antibodies to Ly 1 cells eliminate helper cells preferentially; in contrast, antibodies to Ly 2,3 cells eliminate killer and suppressor cells preferentially (58, 59). Similarly, antibodies have been characterized as recognizing subpopulations of human T cells. The first such demonstration was the finding that certain SLE sera contained antibodies that interacted with a subset of human T cells (23). However, the functions of those cells were not characterized. Subsequently, a rabbit antibody to human T cells was found to selectively kill a subpopulation of human T cells, even though it was

TABLE IV

*Effect of IgG and IgM Fractions from an Active SLE Patient with Anti-T-Cell Antibodies Obtained by Sephadex G-200 Column Chromatography and Requirement of Complement: a Representative Experiment**

Con A-activated culture			Suppressor activity in assay culture			
Source of complement	Fraction of SLE plasma added	Con A activation	MLR‡		PWM§	
			Δcpm±SE	% suppression	Δcpm±SE	% suppression
Autologous fresh plasma	None	−	20,959±1,707		23,792±263	
		+	12,533±1,057	40.2	9,516±475	60.0
	IgG	−	21,868±760		22,357±346	
		+	12,104±1,327	44.6	9,076±224	59.4
	IgM	−	20,121±1,310		24,046±443	
		+	19,702±2,223	1.9‖	21,304±1,526	11.4‖
Heat-inactivated autologous plasma	None	−	22,028±2,032		22,810±655	
		+	12,579±984	42.9	9,352±727	59.0
	IgG	−	21,440±533		25,167±468	
		+	13,087±1,027	39.0	6,291±541	75.0
	IgM	−	22,219±343		21,990±1,726	
		+	13,916±1,388	37.4	11,126±372	49.4

* IgG or IgM fraction (50 μg/ml) was mixed with normal T cells in the presence of culture medium that contained 10% autologous plasma, and incubated with Con A. These Con A-activated cells were then tested for their suppressor activity in the assay culture system.

‡ Same as in Table I.

§ Same as in Table I.

‖ No significant suppression when compared to the response of responder cells that contained T cells precultured without Con A ($P > 0.05$). Others show significant suppression ($P < 0.02$).

capable of binding to all T cells by immunofluorescence (60). Thus, the present observations that anti-T-cell antibodies can kill a population of T cells which acts as suppressor cells, but do not affect a population of T cells that proliferates in response to mitogens, are in agreement with the above cited demonstrations of murine and human T-cell populations. A related study of sera from patients with juvenile rheumatoid arthritis has demonstrated selective killing of a subpopulation of T cells thought to be suppressor cells (61, 62). Whether or not the anti-T-cell antibodies kill a subpopulation of T cells by binding to a unique determinant present only on suppressor cell precursors, or rather kill cells expressing higher densities of T-cell antigen present on some T cells remains to be determined.

Regarding these latter points, our preliminary studies indicate that the brief addition of SLE sera and complement at the beginning of the Con A-activated culture abrogated the development of suppressor cells; however, the brief addition of the SLE sera and complement at the end of the Con A-activated culture did not block the suppressor function. Thus, our evidence could suggest that only suppressor cell precursors are susceptible to the effects of anti-T-cell antibodies.

Anti-T-cell antibodies found to occur spontaneously in New Zealand mice have also been found to preferentially kill suppressor cell precursors. This is true whether the antibodies were studied in vitro or whether they were injected in vivo (24, 63). In patients with SLE, there appears to be an analogous defect. The deficiency in generation of suppressor T cells of SLE patients observed in our previous study (21) can be reproduced in a normal human T-cell population by incubating them with IgM anti-T-cell antibodies obtained from patients with active SLE. These observations suggest the possibility that such antibodies may be the cause of some of the observed T-cell defects in patients with SLE. Nevertheless, a few patients had active disease without antibodies to T cells suggesting that such antibodies do not necessarily circulate in large quantity during active status as has been found in New Zealand mice (64). Whether or

TABLE V

*Effect of Plasma on Generation of Con A-Induced Suppressor T Cells: Study of a SLE Patient before (when Clinically Active) and after (when Clinically Inactive) Therapy**

Con A-activated culture		Suppressor activity in assay culture			
Source of plasma added in culture medium	Con A activation	MLR‡		PWM§	
		Δcpm ± SE	*% suppression*	*Δcpm ± SE*	*% suppression*
Normal individual	−	28,068±1,696		14,659±652	
	+	13,011±404	53.6	6,895±630	53.0
Patient when active	−	30,775±1,367		13,530±329	
	+	32,283±731	−4.8‖	17,120±704	−26.5‖
Normal individual	−	21,774±1,494		12,145±566	
	+	10,368±1,212	52.4	3,580±37	70.5
Patient when inactive	−	19,898±1,318		11,747±893	
	+	19,714±1,878	0.9‖	4,616±593	60.7¶

* Normal T cells were incubated with Con A or without Con A in culture medium supplemented with 10% unheated plasma obtained from either normal individuals or the SLE patient. These activated T cells were then tested for their suppressive capacity in the assay culture system.

‡ Same as in Table I.

§ Same as in Table I.

‖ Significantly different from effect of normal plasma, $P < 0.02$.

¶ Not significantly different from effect of normal plasma, $P > 0.10$.

not such cases are characterized by antibodies bound to, or taken up by cells remains to be determined.

In New Zealand mice, the suppressor T-cell defect and the antibodies to T cells are both present very early in life, making it difficult to determine which one preceded the other (24, 63). SLE patients when inactive do not have easily demonstrable antibodies to T cells. It should therefore be possible in the future by serially

TABLE VI

*Responsiveness of T Cells Remaining after Treatment with Anti-T-Cell Antibodies and Complement**

Treatment of T cells		Responsiveness of T cells remaining after treatment		
Source of plasma	Viable cells after treatment	PHA	Con A	MLR
	% of control‡		*Δcpm ± SE*	
None	100.0	63,239±377	20,837±2,439	24,468±2,283
Normal	97.6	55,555±1,321	14,036±1,945	21,688±3,611
Normal	95.0	59,749±4,310	21,068±3,474	18,641±230
SLE	78.9	52,167±7,155	21,922±522	7,532±1,297§
SLE	78.2	61,273±1,500	29,402±5,763	20,345±1,390
SLE	77.9	59,277±1,816	20,145±3,664	9,045±1,225§
SLE	27.6	57,176±3,063	21,161±2,804	9,554±736§

* Normal T cells were incubated with nonheated plasma either from two normal individuals or from four active SLE patients with anti-T-cell antibodies followed by fresh normal serum as a source of complement, and then tested for their ability to respond to stimulation with PHA, Con A, or allogeneic cells.

‡ Viable cells were determined by trypan blue exclusion following treatment with plasma plus complement. Results are expressed as percentage of viable cells treated with plasma plus complement compared to viable cells treated with complement alone.

§ Significantly different from the responding capacity of T cells treated with complement alone, $P < 0.005$.

testing the blocking activity of SLE sera to determine whether the suppressor T-cell defect precedes the development of anti-T-cell antibodies or vice versa. Alternatively, both may occur simultaneously, as a result of some interaction between environmental factors and the peculiar genetic predisposition of patients with SLE (65).

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