Studies of immune functions of patients with systemic lupus erythematosus. T-cell subsets and antibodies to T-cell subsets.

T Sakane, … , J P Reeves, I Green

Research Article

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Studies of Immune Functions of Patients with Systemic Lupus Erythematosus

T-CELL SUBSETS AND ANTIBODIES TO T-CELL SUBSETS

TSUYOSHI SAKANE, ALFRED D. STEINBERG, J. PATTON REEVES, and IRA GREEN,
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases and Arthritis and Rheumatism Branch, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205

A B S T R A C T   Antibodies to T cells present in the plasma of patients with active systemic lupus erythematosus (SLE) plus complement are able to eliminate concanavalin A-induced suppressor function for the proliferative responses of T cells to allogeneic lymphocytes (MLR) and of B cells to pokeweed mitogen (PWM). Such antibodies were found to be effective in eliminating suppressor function only when T cells were treated before activation; there was no effect when treatment was performed after activation. These studies indicate that the antibodies preferentially interact with a T cell necessary for the generation of suppressor cells, rather than with mature, activated suppressor cells. Studies of individual SLE patients indicate that the same defects observed in SLE T cells were induced in normal T cells by plasma from that patient. Such observations suggest that many T-cell defects associated with active SLE may not be intrinsic T-cell abnormalities, but, rather, secondary effects of anti-T-cell antibodies.

Studies of the T-cell subpopulations responsible for suppression of the MLR and PWM responses indicate that only T_γ cells (T cells bearing receptors for the Fc portion of immunoglobulin [Ig]G) acted as precursors of suppressor cells for the MLR, whereas both T_γ and $T_{non-\gamma}$ cells (T cells not bearing receptors for the Fc portion of IgG) could be activated to suppress the PWM response. Consistent with this observation, SLE anti-T-cell antibodies that preferentially killed T_γ cells preferentially eliminated suppressor cells for the MLR.

INTRODUCTION

Systemic lupus erythematosus (SLE)[1] is a multisystem disease characterized by the production of large quantities of antibodies reactive with self-antigens (1, 2). The immune system of patients with active SLE is characterized by generalized B-cell hyperactivity (3-7) and impaired T-cell function (8-14). The latter may result, at least in part, from a reduction in numbers of T lymphocytes (15-17). Patients with active SLE produce antilymphocyte antibodies, some of which react preferentially with T cells (18-22). Such antibodies may be responsible for the observed loss of T lymphocytes, especially those with a high density of T-cell antigens on their surface membranes (23).

We have previously demonstrated (24) that patients with active SLE have a defect in suppressor T-cell function and that the defect is related to impaired generation of suppressor cells rather than in the response to suppressor signals (which was found to be normal). Others have reported similar observations of impaired suppressor cell activity in patients with SLE (25-27). Moreover, patients with active SLE frequently have antibodies to T cells that are capable of inhibiting the generation of suppressor cells in populations of normal T lymphocytes (28-30). These observations suggest that defects in suppressor function in patients with SLE could be related to the presence of such antibodies.

In view of the subdivision of human T cells on the basis of receptors for the Fc portion of immunoglobulin

[1] *Abbreviations used in this paper*: Con A, concanavalin A; MLR, mixed lymphocyte reaction; PWM, pokeweed mitogen; SLE, systemic lupus erythematosus; SRBC, sheep erythrocytes; T_γ cells, T cells bearing receptors for the Fc portion of IgG; $T_{non-\gamma}$ cells, T cells not bearing receptors for the Fc portion of IgG; T_μ cells, T cells bearing receptors for the Fc portion of IgM.

(Ig)G or IgM (31–34), we have analyzed which of these subpopulations of T cells are normally responsible for suppression of proliferation of responder T cells to allogeneic cells and of responder B cells to pokeweed mitogen. In addition we have performed experiments to determine with which subpopulations the SLE anti-T-cell antibodies react to inhibit the generation of these suppressor functions.

METHODS

Isolation of T cells, non-T cells, and monocytes. Mononuclear cells from the peripheral blood of healthy human donors were isolated by Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) gradient centrifugation. T cells, non-T cells, and monocytes were separated from the mononuclear cells as previously described in detail (35). Briefly, spontaneous rosette formation between human lymphocytes and sheep erythrocytes (SRBC) was performed with neuraminidase-treated SRBC. The rosetting cells were separated on another Ficoll-Hypaque gradient from the nonrosetting cells. Both rosetting and nonrosetting fractions were further purified by a repeated rosette formation with SRBC and subsequent density gradient centrifugation. The doubly purified rosetting cell population was recovered after lysis of SRBC by a Tris-buffered ammonium chloride solution. This population consisted of >95% T cells as determined by rerosetting, and will be referred to as T cells. The doubly purified nonrosetting population was depleted of monocytes by removal of cells adhering to Petri dishes. Monocytes were obtained by collecting the cells adhering firmly to the dishes. The nonadherent, nonrosetting cell population consisted of B cells (42.4±3.4%), L cells (54.5±3.4%) determined by rerosetting, and was contaminated with <1% of T cells (the criteria used for these assignments of cell type are cited by Sakane and Green [35]). We will refer to this population as non-T cells. More than 95% of "monocyte" preparations were monocytes as judged by morphology after Giemsa staining.

Fractionation of T cells. Purified T cells were subjected to further fractionation into T cells bearing receptors for the Fc portion of IgG (T_γ) cells and T cells not bearing receptors for the Fc portion of IgG ($T_{non-\gamma}$) by employing the preferential ability of T_γ cells to form rosettes with IgG-coated ox erythrocytes (36). These rosetting cells were separated from nonrosetting cells by centrifugation over Ficoll-Hypaque. The T_γ cell fraction contained 80–85% Fc(IgG)-rosetting cells, whereas in the $T_{non-\gamma}$ cell fraction, the proportion of cells forming Fc(IgG)-rosettes was <1%.

Patients and source of plasma. Patients satisfying the diagnostic criteria of the American Rheumatism Association for SLE were admitted to the Arthritis Branch of the National Institute of Arthritis, Metabolism, and Digestive Diseases at the Clinical Center, National Institutes of Health. Plasmas were obtained from patients with active disease before treatment. Patients with inactive disease had previously been treated with corticosteroids and occasionally with azathioprine; these patients had not received such treatment for many weeks or months and were untreated 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, 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 using flow microfluorometry as previously described (23). All the SLE plasma used had been fresh-frozen and had never been previously thawed. Normal fresh-frozen plasma were obtained from healthy adults (between 16 and 48 yr old). All plasma were centrifuged at 105,000 g for 2 h at 4°C to remove aggregated materials before use. (Individual patients are designated by a capital letter.)

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 from SLE patients was incubated with 2.5–3.0 × 10⁸ 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.

Preparation of IgG and IgM fractions of plasma by Sephadex G-200 column chromatography. Plasma was precipitated with 50% ammonium sulfate, dialyzed against buffer (0.2 M borate buffer, pH 8.0), and applied to a 1.5-m long Sephadex G-200 (Pharmacia Fine Chemicals) column. Individual fractions were collected and the optical 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 using immunoplates impregnated with highly purified antisera specific for human IgG or IgM. The IgM and IgG peaks were separately pooled and concentrated. Individual fractions were dialyzed overnight against phosphate-buffered saline, pH 7.2, before use in in vitro studies.

Experimental design of suppression studies. Suppressor T cells were generated by concanavalin (Con A, Pharmacia Fine Chemicals) A activation in a first culture. These cells were added to responder cells in a second assay culture system that was stimulated with either mitogens or allogeneic cells. The suppressor cells from the first culture and the responder cells in the second culture were from the same individual (24, 28, 37). In detail, 3 × 10⁶ normal T cells (unfractionated T cells, T_γ cells, and $T_{non-\gamma}$ cells) were incubated in 3 ml culture medium, RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.), supplemented with 100 U penicillin/ml, 100 μg streptomycin/ml, 2 mM L-glutamine, 25 mM Hepes buffer, and 10% fetal bovine serum (Microbiological Associates, Walkersville, Md.) with Con A, 30 μg (Con A-activated T cells), or without Con A (nonactivated control T cells) at 37°C in a humidified 5% CO₂/95% air environment. To both nonactivated control and Con A-activated cultures, 0.2 × 10⁶ mitomycin-treated (Sigma Chemical Co., St. Louis, Mo.) monocytes were added (38). 60 h later, the cells were harvested, washed four times, treated with mitomycin, and then tested for their suppressor activity in the second assay culture system. For this assay, responder cells were obtained 3 d later from a new bleeding of the same individual who originally provided the suppressor cells. Stimulatory mitogens or allogeneic stimulating cells were added to 1 × 10⁵ responder cells in microtiter plates; in addition, 1 × 10⁵ mitomycin-treated Con A-activated or nonactivated control T cells from the first culture were added. To fully develop the suppressor activity by the Con A-activated cells, mitomycin-treated monocytes (5,000 per culture) were also added to the second culture system (26). Where T lymphocytes were used as responder cells, they were stimulated by either phytohemagglutinin (The Wellcome Research Laboratories, Beckenham, England), 0.1 μg/ml, or 1 × 10⁵ mitomycin-treated allogeneic stimulating cells. When non-T lymphocytes were used as responder cells, pokeweed mitogen (PWM; Grand Island Biological Co.), 2 μg/ml, was the

stimulant in the culture. As the non-T-cell response to PWM is clearly T-cell dependent, freshly prepared autologous T cells that had been treated with mitomycin were also added (5×10^4 per culture). T-cell cultures stimulated by phytohemagglutinin were harvested on day 4; B-cell cultures stimulated with PWM were harvested on day 6. In both cases, DNA synthesis in the second culture period was assayed by addition of 1 μCi of [methyl-^{3}H]thymidine (5 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) to the culture for the final 20 h of the second culture period.

The degree of suppression was calculated with the following formula:

previously found that active SLE patients frequently, but not always, have defects in suppressor cell generation (24). Furthermore, we have found that plasma from many active SLE patients can inhibit the generation of suppressor cells in T-cell populations obtained from normal individuals (28). We therefore asked whether the pattern of suppressor activity observed in lymphocytes from particular SLE patients could be imparted to normal T cells by the plasma from these same patients. We found that patients with defects in suppres-

$$
\% \text{ suppression} = \left(1 - \frac{\substack{\text{Mean cpm of stimulated cultures containing Con A-activated T cells} \\ - \text{ Mean cpm of unstimulated cultures containing Con A-activated T cells}}}{\substack{\text{Mean cpm of stimulated cultures containing nonactivated T cells} \\ - \text{ Mean cpm of unstimulated cultures containing nonactivated T cells}}} \right) \times 100.
$$

The basic suppressor system was then perturbed by a number of different procedures. In some experiments unfractionated T cells were treated with SLE plasma plus complement either before or after the first culture; in some cases they were treated with SLE plasma plus complement both before and after the first culture. In other experiments, adsorbed plasma or IgG or IgM fractions were substituted.

Treatment of normal T cells with SLE plasma plus complement. 1×10^7 normal T cells before or after activation by Con A in 1 ml RPMI 1640 were mixed with 1 ml SLE plasma (either unadsorbed or adsorbed with normal T cells or non-T cells); these mixtures were kept at 4°C for 1 h. These T cells were then washed, resuspended in 0.5 ml RPMI 1640, and incubated with 0.5 ml fresh normal human serum as a complement source at room temperature for another 3 h. Thereafter these cells were washed three times, resuspended in culture medium, and viable cell yield was determined by trypan blue exclusion. The percentage decrease in the number of viable cells resulting from such treatment was also calculated (see formula below), based on the original viable cell number present before such treatment.

When normal T cells were treated with the IgM or the IgG fraction of SLE plasma (prepared by Sephadex G-200 column chromatography), the same above procedure was employed except that 1 mg of the fraction was added to 1×10^7 normal T cells in a final volume of 1 ml, instead of addition of the plasma.

Evaluation of specificities of SLE anti-T-cell antibodies against normal human T-cell subsets. To determine whether or not SLE anti-T-cell antibodies could preferentially kill human T-cell subsets, normal T cells fractionated into T$_\gamma$ and T$_{non-\gamma}$ cells were examined for their susceptibility to killing by the anti-T-cell antibodies. Thus, 2×10^5 normal T cells in 0.1 ml RPMI 1640 were mixed with 0.1 ml plasma from either SLE patients or normal individuals at 4°C for 5 h. These T cells were then incubated with 0.05 ml fresh normal human serum as a complement source at room temperature overnight. Thereafter viable cell yield was determined by trypan blue exclusion and the percent of cell killing was calculated with the following formula:

sor cell generation had anti-T-cell antibodies in their plasma which caused the same defects in a normal T-cell population. A representative group of patients is shown in Table I. Patient A with active SLE, and with defects in generation of suppressor cells for both the allogeneic lymphocyte (MLR) and PWM responses, had anti-T-cell antibodies that induced the same defects in normal T cells. However, when the same patient A became inactive, normal suppressor function of her lymphocytes, as well as a lack of inhibition of suppressor cell generation in normal T cells by her plasma was observed. Patient K also had inactive SLE and normal suppressor function. She did not have antibodies that inhibited suppressor cell generation of normal T cells. Of particular interest was patient U. Her T lymphocytes had a defect in the generation of suppressor function for the MLR response, but not for the suppressor function of the PWM response. Exposure of normal T lymphocytes to her plasma induced in these lymphocytes the same pattern of suppressor function defects as was observed in the lymphocytes of patient U.

Antibodies interact with cells necessary for the generation of suppressor cells rather than with mature suppressor cells. The first series of experiments involved attempts to inactivate suppressor T cells from normal donors with SLE anti-T-cell antibodies. Treatment of normal T cells with anti-T-cell antibodies plus complement was performed either before or after Con A activation, or at both times. A representative experiment is shown in Table II. Plasma containing anti-T-cell antibodies plus complement added to normal T cells before Con A activation markedly inhibited the generation of functional suppressor cells. In contrast,

$$
\% \text{ cell killing} = \left(1 - \frac{\text{Number of viable T cells after treatment with plasma plus complement}}{\text{Number of viable T cells after treatment with complement alone}} \right) \times 100.
$$

RESULTS

Antibodies from SLE patients confer on normal T cells the defects found in these SLE patients. We have

treatment of T cells at the end of the Con A activation step had no effect on suppressor cell function. When the treatment was performed both at the beginning and

TABLE I
Lymphocyte Suppressor Function of Each Individual Patient with SLE is Associated with the Effect of the Plasma on Suppressor Generation of Normal T Cells

	Con A-induced suppression	
	MLR	PWM
	% suppression	
Normal		
Lymphocyte suppressor function*	52.2	40.2
Plasma effect on normal cells	51.1 (54.6)	43.0 (43.5)‡
Active SLE, patient A		
Lymphocyte suppressor function	13.8	17.8
Plasma effect on normal cells	20.0 (47.7)	4.2 (54.5)
Inactive SLE, patient A		
Lymphocyte suppressor function	62.6	81.5
Plasma effect on normal cells	52.6 (47.7)	53.5 (54.5)
Inactive SLE, patient K		
Lymphocyte suppressor function	54.7	51.5
Plasma effect on normal cells	45.5 (48.7)	51.7 (46.8)
Inactive SLE, patient U		
Lymphocyte suppressor function	21.9	45.4
Plasma effect on normal cells	11.5 (53.6)	40.5 (44.3)

* Con A-induced T cells from 27 normal individuals gave 56.2±2.4% suppression (mean±SE) for MLR and 53.1±3.1% suppression for PWM response.
‡ Values in parentheses show suppression produced by Con A-activated normal T cells which had received prior treatment with fetal bovine serum and complement or normal plasma plus complement.

TABLE II
Effect of Treatment with a Single SLE Plasma Containing Anti-T-Cell Antibodies before and/or after First Culture on the Generation of Con A-induced Suppressor T Cells

First culture			Second culture					
Treatment with plasma plus complement			Thymidine incorporation in response to					
Before first culture*	After first culture*	Con A	PHA‡		MLR		PWM	
			Δ cpm±SE	% suppression	Δ cpm±SE	% suppression	Δ cpm±SE	% suppression
0	0	−	14,111±1,331		10,182±204		13,607±528	
		+	6,822±445	51.7	4,327±169	57.5	5,377±491	60.5
+	0	−	13,257±208		10,613±1,056		15,374±854	
		+	13,440±650	−1.4	11,265±1,434	−6.1	15,649±447	−1.8
+	+	−	15,612±2,159		11,057±819		15,596±1,479	
		+	14,856±1,937	4.8	9,684±687	18.4	16,534±512	−6.0
0	+	−	19,303±1,136		9,910±459		14,488±1,362	
		+	5,862±748	69.6	4,023±426	59.4	6,018±56	68.5

* Normal T cells were treated with SLE plasma plus complement before and/or after Con A activation in the first culture. These T cells were then tested for their suppressive ability with autologous responder cells.
‡ Phytohemagglutinin.

at the end, the result was not much different from that obtained when treatment occurred only at the beginning. As a result, we omitted the double treatment data from the remainder of the studies.

A summary of studies of plasma from 14 patients with SLE is shown in Table III. Plasma from normal donors had no effect upon suppressor cell function. Plasma from patients with inactive SLE or active SLE without anti-T-cell antibodies did not markedly inhibit the generation of suppressor function. In contrast, plasma containing anti-T-cell antibodies from five active patients markedly inhibited the generation of suppressor function. This effect occurred when the antibodies and complement were added before Con A activation; however, again there was no significant effect if the T cells were exposed to these plasma at the end of the Con A activation step (Table III). It was also noted that there was a 37.2±6.1% decrease in viable cell number when these five plasma containing anti-T-cell antibodies were added before Con A activation, but only a 7.5±7.6% decrease in viable cell number when added at the end of the culture. These observations suggested that SLE anti-T-cell antibodies could kill a cell necessary for the generation of suppressor cells, but could not easily kill mature suppressor cells.

In the course of the present studies, a curious phenomenon (as already noted in Table I with plasma from patient U) was observed with plasma obtained from patients U and L. Plasma of patient U, when inactive, was able to inhibit the generation of suppresor function of normal T cells for the MLR (Table IV). In contrast, her plasma did not inhibit the generation of suppressor function for PWM-induced proliferation. Furthermore, plasma from another patient, L, with mildly active disease, exhibited the same dichotomous effect (Table IV). Her plasma also inhibited the generation of suppressor function for the MLR, but not for the response to PWM. These observations led us to prospectively evaluate the T-cell subpopulations mediating the suppression of the two functions.

Role of T_γ and $T_{non-\gamma}$ cells in the generation of suppressor function for the MLR and PWM proliferative responses. Unfractionated T cells and T cells fractionated into T_γ and $T_{non-\gamma}$ populations obtained from normal individuals were tested for their ability to be activated by Con A to mediate suppression of the T-cell proliferative response to MLR or the non-T-cell proliferative response to PWM. Results of such experiments are shown in Table V. We found that unfractionated T cells activated by Con A led to suppression of both

TABLE III

Effect of Treatment with Plasma from Patients with Active or Inactive SLE before or after First Culture on Generation of Con A-induced Suppressor T Cells: Summary

| | First culture | | | Second culture | |
| | Plasma | | Treatment with plasma plus complement | | Con A-induced suppression | |
Source	Number tested	Before first culture*	After first culture*	MLR	PWM
				% suppression ±SE	
None‡		0	0	57.4±4.5	53.0±2.6
Normal	5§	+	0	51.6±2.0	52.4±4.7
		0	+	58.5±4.1	50.0±4.4
Inactive SLE	6§	+	0	41.6±5.0	49.3±3.0
		0	+	51.1±2.5	54.7±2.6
Active SLE without anti-T-cell antibodies	3§	+	0	56.1±9.3	52.6±1.3
		0	+	50.4±3.9	62.8±4.2
Active SLE with anti-T-cell antibodies	5§	+	0	8.8±3.4‖	8.5±13.4‖
		0	+	60.0±8.0	50.7±3.8

* Same as Table II.
‡ This control group represents the results from 12 experiments performed simultaneously to those with plasma in which the cells of the first culture were not treated with any plasma.
§ The number of individual plasma donors tested is shown.
‖ Significantly different from a group where T cells were treated with normal plasma plus complement ($P < 0.02$) and significantly different from a control where T cells were not treated with plasma plus complement ($P < 0.001$).

TABLE IV
Differential Effect of SLE Plasma on the Killing of Precursors of Suppressor T Cells for Either the MLR or the PWM Response

	First culture		Second culture	
	Treatment with plasma plus complement		Con A-induced suppressor activity	
Plasma source	Before first culture*	After first culture*	MLR‡	PWM§
			% suppression	
Active SLE,	0	0	48.7	46.8
patient L	+	0	−1.4	49.2
	0	+	52.9	59.7
Inactive SLE,	0	0	53.6	44.3
patient U	+	0	11.5	40.5
	0	+	47.0	49.5

* Same as Table II.
‡ Same as Table II.
§ Same as Table II.

reactions. Similarly, T_γ cells led to suppression of both reactions, whereas the $T_{non-\gamma}$ cells activated by Con A failed to suppress the MLR; however, they were capable of suppressing the PWM-induced proliferation.

In the above experiments, a single dose of suppressor cells (10×10^4) from the first culture was used to test for suppressor activity. In the next experiment, varying numbers of Con A-activated T_γ or $T_{non-\gamma}$ cells were employed; a dose-response curve was constructed by plotting the number of Con A-activated cells added to the assay culture vs. the percent suppression (Fig. 1). Only T_γ cells could suppress the MLR; four times the

TABLE V
Differential Suppressor Activity of T_γ and $T_{non-\gamma}$ Cells: Using T Cells from Six Different Normal Individuals

	First culture	Second culture	
T-cell fraction used		MLR‡	PWM§
		Mean % of suppression ±SE	
Unfractionated T cells		46.5±4.2	74.0±4.2
T_γ cells		56.1±3.2	77.7±4.5
$T_{non-\gamma}$ cells		9.9±3.3	49.5±4.9

* Normal T cells, unfractionated or fractionated, were pre-cultured with or without Con A in the first culture. These T cells were assayed for their suppressor activity in the second culture.
‡ Unfractionated T cells were used as responder cells in the second assay culture.
§ Non-T cells were used as responder cells in the second assay culture.

usual number (10^5) of $T_{non-\gamma}$ cells were ineffective. With regard to the suppression of PWM response, both T_γ and $T_{non-\gamma}$ cells could suppress; however, T_γ cells were more effective than $T_{non-\gamma}$ cells. Of particular interest, at low cell numbers, Con A-activated cells tended to be stimulatory rather than suppressive of the PWM response (Fig. 1).

Effect of selected SLE anti-T-cell antibodies on subpopulations of T cells. In view of the observations described above that (a) some SLE anti-T-cell antibodies interfere with the generation of suppressor cells for the MLR but not for PWM responses, and (b) that the MLR is suppressed only by T_γ cells, whereas the PWM response is suppressed by both T_γ and $T_{non-\gamma}$ cells, we further studied the effect of plasma from patients
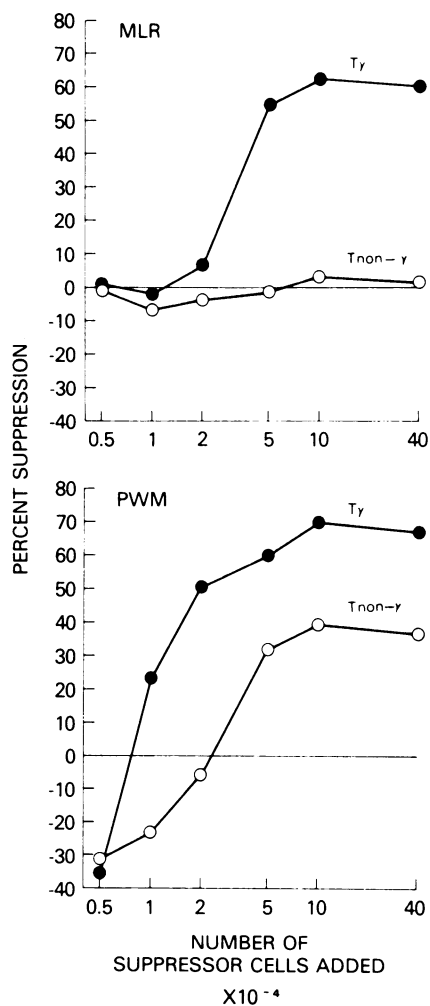

FIGURE 1 Dose-response analysis of suppressive ability of fractionated T cells. Responder cells, 1×10^5, were stimulated with either MLR or PWM in cultures containing increasing numbers of Con A-activated T_γ or $T_{non-\gamma}$ cells.
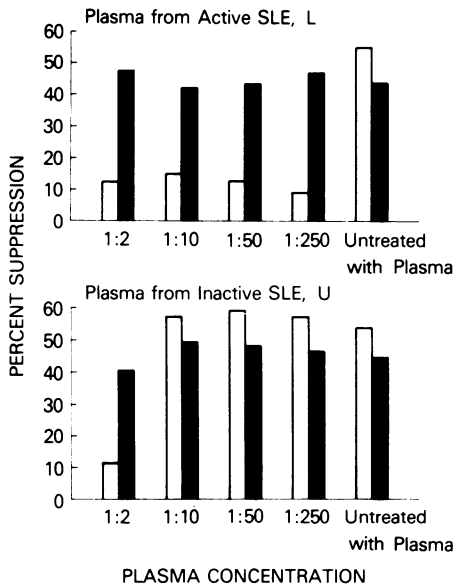
FIGURE 2 Dose-response analysis of anti-T-cell antibodies obtained from SLE patients L and U. Normal T cells were treated with various concentrations of SLE plasma and then exposed to normal serum as a complement source. After Con A activation of these cells, their suppressive ability was tested in the second culture. □, percent suppression of MLR; ■, percent suppression of PWM response.

L and U, which could block the generation of suppressor cells for the MLR, but not for the PWM response. The first study was a dose-response analysis of each anti-T-cell antibody and the effect upon suppressor cell generation (Fig. 2). Plasma from mildly active patient L, even when diluted to 1:250, still had a profound inhibiting effect on the generation of suppressor cells for the MLR. Plasma from inactive patient U, lower panel of Fig. 2, only inhibited MLR suppressor cell generation at a dilution of 1:2.

When the specificities of those two plasma against normal human T-cell subsets were further examined, active plasma from patient L, in which anti-T-cell antibodies had already been demonstrated by indirect immunofluorescence, could kill 28% of whole unfractionated T cells by trypan blue exclusion. This plasma had preferential ability to kill T_γ cells of normal donors (65% killing for T_γ cells and 17% for $T_{non-\gamma}$ cells). Inactive plasma from patient U could kill only 7% of unfractionated normal T cells by trypan blue exclusion. However, when fractionated normal T cells were used, 74% of T_γ cells and none of the $T_{non-\gamma}$ cells could be killed by this plasma. Thus, there was a correlation between the subpopulation killed and the suppressor function abrogated. That is, these two plasma, which were preferentially cytotoxic for T_γ cells, preferentially interfered with the generation of suppressor function for the MLR, and T_γ-mediated function. In contrast,

most active SLE anti-T-cell antibodies killed both T_γ and $T_{non-\gamma}$ cells and interfered with generation of suppressor cells for both the MLR and the PWM. To be specific, the average percent of killing by four separate SLE plasma containing anti-T-cell antibodies (excluding patient L) was as follows: $48.3 \pm 5.5\%$ of unfractionated T cells, $53.4 \pm 9.1\%$ of T_γ cells, and $37.1 \pm 6.4\%$ of $T_{non-\gamma}$ cells.

The possible specificity of the factor present in the plasma of patients L and U responsible for the elimination of suppressor cells for the MLR but not for the PWM response was further studied by adsorption of these plasma with either normal T cells or non-T cells (Fig. 3). Adsorption with T cells almost completely removed the activity of the SLE plasma. In contrast, adsorption with non-T cells did not remove the activity.

When plasma from these two patients were fractionated by Sephadex G200 chromatography, the IgM, but not the IgG, fraction contained the active antibody that prevented the generation of suppressor cells for only the MLR, but not for the PWM response (Table VI).

DISCUSSION

We have previously reported that anti-T-cell antibodies obtained from patients with active SLE are capable of inhibiting the generation of suppressor T cells for proliferation in response to stimulation with both MLR and PWM (28). In the present study we found that this inhibition occurs when the antibody treatment is performed before activation, whereas it is ineffective after activation. Thus, the SLE anti-T-cell antibodies interfere with a cell necessary for the generation of suppressor T cells, probably by killing suppressor cell precursors, although it is possible that another T cell, such as an initiator cell, is both necessary for suppressor cell activation and killed by the anti-T-cell antibodies. When patients with SLE were studied with regard to (a) their own T-cell function and (b) effects of their plasma on normal T-cell function, the same defect(s) observed with the patient's T cells were induced by treating normal T cells with their plasma. Thus the presence of antibodies, rather than intrinsic T-cell abnormalities, may be responsible for the T-cell defects observed in patients with active SLE.

Although most anti-T-cell antibodies from patients with active SLE eliminated suppressor function for both the MLR and PWM responses, one patient with mildly active SLE and one with inactive disease were found to have anti-T-cell antibodies that preferentially eliminated suppressor cell precursors for the MLR, but did not interfere with the generation of suppressor T cells for the PWM response. We found that such antibodies were of the IgM class, were adsorbed with T cells (but not non-T cells), and that they preferentially killed T_γ cells as compared with $T_{non-\gamma}$ cells. The ob-
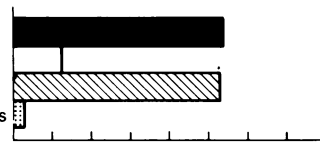
FIRST CULTURE   SUPPRESSOR ACTIVITY IN ASSAY CULTURE
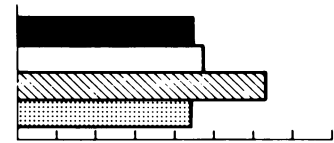
PLASMA SOURCE   MLR   PWM

Active SLE,   None
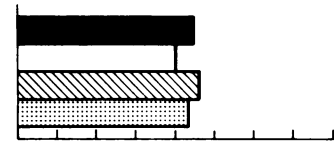   L   Unabsorbed
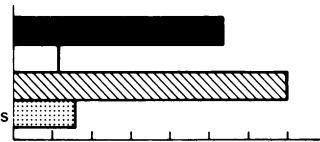   Absorbed with Normal T Cells
               Absorbed with Normal Non-T Cells

Inactive SLE, None
    U   Unabsorbed
               Absorbed with Normal T Cells
               Absorbed with Normal Non-T Cells

               0   20   40   60   80   0   20   40   60   80

                              PERCENT SUPPRESSION

FIGURE 3   Effect of SLE plasma adsorbed with normal T cells or non-T cells on the generation
of suppressor T cells. Plasma from two SLE patients, L and U, which could inhibit the suppressor
cell generation for the MLR but not for the PWM response, was first adsorbed with either normal
T cells or non-T cells. The adsorbed plasma to be studied was mixed with normal T cells.
Thereafter these mixtures were exposed to complement. Such treated T cells were activated
with Con A, and then these Con A-activated cells were tested for their suppressor activity.

servation that the antibodies were of the IgM class strongly suggested that they were not interfering with T-cell functions by binding to Fc receptors on the T_γ cells; however, we have not formally demonstrated that they bound to the T_γ cells through the antibody-combining site. The finding that selected anti-T-cell antibodies had specificity for both T_γ cells and precursors of suppressor T cells for the MLR but not for the PWM

TABLE VI
Effect of IgG and IgM Fractions of SLE Plasma Obtained
by Sephadex G-200 Column Chromatography on the
Generation of Suppressor T Cells*

Source of plasma fractions	Fractions added in first culture	Suppressor activity in second culture	
		MLR‡	PWM§
		% suppression	
Active SLE, patient L‖	None	65.5	69.6
	IgG	52.5	59.9
	IgM	6.2	62.0
Inactive SLE, patient U‖	None	95.1	35.2
	IgG	91.5	40.1
	IgM	26.5	36.8

* Normal T cells were treated with either IgG or IgM fraction of SLE plasma plus complement and activated with Con A. These Con A-activated T cells were then tested for their suppressor activity in the second culture.
‡ T cells were used as responder cells.
§ Non-T cells were used as responder cells.
‖ These experiments were performed on different days and employed different donors for normal cells.

responses led us to examine the nature of the T-cell subsets responsible for suppression of the MLR and the PWM.

Several studies have suggested that subpopulations of human T cells can be separated on the basis of receptors for the Fc portion of different immunoglobulin classes (isotypes) (27, 31–34, 36). Moretta and colleagues initially felt that T_γ cells were specific for the function of suppression, whereas T cells bearing receptors for the Fc portion of IgM (T_μ cells) were specific for the function of help (31, 34). Those studies examined the effect of the different T-cell subpopulation on B-cell functions. Subsequently, it has been found in an assay of Con A-activated suppressor cells that T_μ cells may under certain circumstances function as suppressor cells (32). In the present study, we found that T_γ cells were more effective than $T_{non-\gamma}$ cells at suppressing both the PWM and MLR reactions. $T_{non-\gamma}$ cells significantly suppressed the PWM response; however, they were unable to suppress the MLR. These studies helped to explain the effects of the two selected SLE antibodies described above. Those anti-T-cell antibodies capable of killing only T_γ cells interfered only with the generation of suppressor cells for the MLR. In contrast, those antibodies capable of killing both T_γ and $T_{non-\gamma}$ cells interfered with the generation of suppressor cells for both responses. Because T_γ cells alone are involved in the generation of suppressor cell function for the MLR, the antibodies that preferentially killed those cells would be expected to preferentially eliminate the function mediated by those cells. Whether or not T_γ cells alone are responsible for suppression of all T-cell functions remains to be determined; it is

clear, however, that the non-T-cell proliferative response to PWM is suppressible by both T_γ and $T_{non-\gamma}$ cells. This helps to explain previous reports which claimed that both T_γ cells and T_μ cells could act as suppressor cells (31, 32, 34).

Furthermore, it was noted that small numbers of T_γ and $T_{non-\gamma}$ cells led to stimulation of the PWM response rather than suppression. Such an observation underlines the importance of dose-response curves before deciding that a particular subset of T cells subserves a particular function. We presume that there are both helper and suppressor subsets of T_γ cells to explain these observations. This idea is also supported by the finding that T cells treated with SLE anti-T-cell antibodies plus complement and activated with Con A often produced an increase in the PWM response rather than a decrease. However, further dose-response studies using SLE anti-T-cell antibodies plus Fc (IgG) receptor separation will be necessary to discriminate between helper and suppressor T_γ cells.

Previous studies of SLE anti-T-cell antibodies suggest that some may be of the IgG class (18, 20, 22, 30). The present study measured the effects of complement-mediated killing of T cells. It has previously been shown in mice that IgM antibodies to T cells are 500 times more efficient at complement-mediated lysis than are IgG antibodies; therefore, the activity of the IgM antibodies might be the only one observed despite the presence of large amounts of anti-T-cell antibodies of the IgG class (39).

Furthermore, at least one mechanism by which IgG antibodies eliminate T cells is antibody-dependent cellular cytotoxicity (ADCC) (22). Because in the present study only highly purified T-cell populations were treated with SLE antibodies, effector cells for antibody-dependent cellular cytotoxicity might not have been present in sufficient numbers to mediate T-cell killing. Thus, the present studies do not deny the presence of IgG anti-T-cell antibodies in SLE. However, since they were designed to study complement-mediated T-cell killing, they may have preferentially selected for IgM antibody activity.

Recent studies have demonstrated that sera from patients with juvenile rheumatoid arthritis contain antibodies capable of recognizing suppressor cells (40, 41). In addition the patients with such antibodies lack suppressor cells and have increased numbers of immunoglobulin-secreting cells (41). Thus, in both juvenile rheumatoid arthritis and SLE the presence of the antibodies and the loss of suppressor cells correlated with disease activity. Circumstantially, these results suggest that in some patients disease may be initiated by some factor or factors which lead to formation of anti-T-cell antibodies and subsequent loss of suppressor cells. Once formed, anti-T-cell antibodies would be capable of eliminating cells that would serve to reduce

B-cell activity. Thus, a self-perpetuating cycle would occur with continued high level production of auto-antibodies and continued loss of the very cells that serve to suppress such excessive antibody production.

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