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Studies of immune functions of patients with systemic lupus erythematosus: antibodies to desialized, rather than intact, T cells preferentially bind to and eliminate suppressor effector T cells.

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Research Article

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Studies of Immune Functions of Patients with Systemic Lupus Erythematosus

ANTIBODIES TO DESIALIZED, RATHER THAN INTACT, T CELLS PREFERENTIALLY BIND TO AND ELIMINATE SUPPRESSOR EFFECTOR T CELLS

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ABSTRACT Patients with systemic lupus erythematosus (SLE) were found to have in their plasma antibodies specific for desialized T cells. Adsorption studies with intact or desialized T cells indicated that SLE anti-T cell antibodies consisted of two populations with different target cell specificities, one capable of recognizing unique determinants on desialized T cells and another able to bind to both intact and desialized T cells. Normal T cells did not remove the antibodies specific for desialized T cells. Moreover, the antibodies to desialized T cells were not removed by adsorption with either desialized non-T cells or desialized erythrocytes. Thus, the antibodies to desialized T cells recognize a determinant that is unique to a T cell subset and also includes a sugar. Inhibition studies with various sugars indicated that lactose was the most potent inhibitor of antibody binding. The anti-desialized T cell antibody appears to recognize a T cell determinant which includes lactose, probably in the form of a β-galactosyl residue, but which also includes additional T cell determinants.

The antibodies to desialized T cells were found to bind preferentially to concanavalin A-induced autorosetting T cells, which had been already demonstrated to contain suppressor effector cells. Indeed, such antibodies were effective in eliminating suppressor effector function without interfering with T cells necessary for such activation (such as precursor or inducer cells). Finally, studies of patients with SLE yielded a highly significant correlation ($r = 0.92$) between impaired suppressor effector function of their cells and the presence of antibodies to desialized T cells in their plasma.

INTRODUCTION

Recent studies of our own (1–3) and those of others (4–11) have demonstrated in both humans and mice that masking the cell surface by sialic acid occurs with T cell maturation; relatively immature T cells have a low sialic acid content and include cells involved in suppressor effector functions. Helper T cells consist of more mature T cells which have abundant sialic acid residues on the cell surface.

Numerous reports have documented the occurrence of anti-T cell antibodies in active systemic lupus erythematosus (SLE)[1] (12–22). A subset of such antibodies preferentially interfere with a T cell necessary for the generation of suppressor cell activity, probably by killing suppressor precursor cells or their initiator cells

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[1] *Abbreviations used in this paper*: Con A, concanavalin A; PBS, phosphate-buffered saline; SLE, systemic lupus erythematosus.

or both (20, 21). Analysis of natural occurring anti-T cell antibodies of New Zealand mice has shown that anti-T cell antibodies directed at intact T cells, differ from those directed at desialized T cells (23). The present studies were carried out to investigate the possibility that some SLE anti-T cell antibodies might preferentially interact with desialized T cells. We found that such SLE anti-T cell antibodies exist. Moreover, they specifically bind to and kill functional suppressor effector cells. These antibodies to desialized T cells recognize a different T cell subset from those to intact T cells; the latter recognize a cell necessary for the generation of suppressor cells, but not mature suppressor cells (21).

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 centrifugation. T cells, non-T cells, and monocytes were separated from the mononuclear cells as previously described in detail (20, 21, 24). Briefly, spontaneous rosette formation between human lymphocytes and sheep erythrocytes was performed with neuraminidase treated sheep erythrocytes. The rosetting cells were separated on another Ficoll-Hypaque gradient from the nonrosetting cells. Both the rosetting and nonrosetting cell fractions were further purified by repeated rosette formation with sheep erythrocytes and subsequent density gradient centrifugation. The doubly purified rosetting cell population was recovered after lysis of sheep erythrocytes 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 is referred to as non-T cells and contains B lymphocytes, monocytes, and possibly other cell types. The percentage of T cell contamination in the non-T cell population was <1%, as determined by rerosetting. Monocytes were obtained by incubating the non-T cells on petri dishes for 2 h at 37°C, and collecting the cells adhering firmly to the dishes. Monocyte preparations consisted of 95% cells that were identified as monocytes after Giemsa staining.

Patients and source of lymphocytes and plasma. Adult patients satisfying the diagnostic criteria of the American Rheumatism Association for SLE were followed by the Shimane Medical University Hospital and by the Arthritis Branch of National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases at the Clinical Center, National Institutes of Health. Clinical activity was assessed at the time of blood drawing 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 (20, 21, 25). The active patients in this study had at least three of the above criteria for activity; in addition, they all had high titers of antibodies to native DNA (20, 21, 25). Lymphocytes and plasma from the patients with active disease were obtained for in vitro studies before any treatment. Inactive patients had been previously treated with corticosteroids and occasionally with immunosuppressive drugs, but were not receiving such treatment at the time of study. All SLE plasma used had been fresh frozen and had not been previously thawed. All plasma were centrifuged at 105,000 *g* for 2 h at 4°C to remove aggregated materials before use.

Treatment of T cells with enzymes. 1×10^7 normal T cells were suspended in 1 ml of Hanks' balanced salt solution containing 10 mM Hepes buffer, pH 7.0. 1 ml of the cell suspension was mixed with 0.1 U of Vibrio comma neuraminidase (*N*-acyl-neuraminyl-glycohydrolase, 3.2.1.18 from *Vibrio comma* (Cholerae); Behring Institut, Marburg, West Germany) for 1 h at 37°C. More than 98% of the T cells after neuraminidase treatment were found to be stained with fluorescein-conjugated peanut agglutinin (E·Y Laboratories, Inc., San Mateo, CA), which indicates that sialic acid residues were almost completely removed from the cell surfaces. T cells were treated with either papain (Sigma Chemical Co., St. Louis, MO) (60 μg/ml of the cell suspension) or trypsin (Difco Laboratories, Inc., Detroit, MI) (20 and 200 μg/ml of the cell suspension) under similar conditions. Pronase (pronase-P; Kaken Chemical Co., Tokyo, Japan) (50 and 200 μg/ml of the cell suspension) treatment was performed at room temperature for 10 min. After treatment with various enzymes, these cells were washed three times in phosphate buffered saline (PBS).

Adsorption of SLE plasma with normal T cells, non-T cells, or erythrocytes, either intact or neuraminidase treated. SLE plasma was incubated with various numbers of packed T cells or non-T cells, either intact or neuraminidase treated ($1–10 \times 10^7$ cells/ml plasma) at 4°C overnight. Adsorption of the plasma with human erythrocytes was performed by adding 1 ml plasma to 1–5 ml packed erythrocytes (either intact or neuraminidase treated), followed by incubation at 4°C overnight. The residual capacity of supernatant plasma obtained by centrifugation to bind to intact T cells or neuraminidase treated desialized T cells obtained from normal individuals was determined by indirect immunofluorescence staining.

Immunofluorescence staining. Normal T cells, either intact or neuraminidase treated, or these cells further treated with proteolytic enzymes, were suspended in PBS at a concentration of 1×10^7/ml. The cell suspension was mixed with an equal volume of heat-inactivated plasma from either SLE patients or normal individuals (unadsorbed or adsorbed with intact or desialized cells) in a total volume of 200 μl; subsequently the mixtures were incubated for 1 h at 4°C. After incubation, the cells were washed three times in cold PBS, resuspended in 75 μl of fluorescein isothiocyanate-labeled $F(ab')_2$ fragment of rabbit anti-human IgG or IgM (F/P ratios 3.0–4.5 mg/g; N.L. Cappel Laboratories, Inc., Cochranville, PA). All fluorescein-labeled antisera were ultracentrifuged for 30 min at 105,000 *g* before use to eliminate aggregates. The cells were incubated for 30 min at 4°C with fluorescein-labeled antisera, and washed three times in PBS. For all experiments, at least 500 cells were examined under a fluorescence microscope (Nihon Kogaku Industries, Inc., Tokyo, Japan).

Assay of suppressor cell function. Suppressor T cells were generated by concanavalin A (Con A) activation in a first culture as previously described (20, 21, 25). These cells were added to responder T cells in a second assay culture system that were stimulated with allogeneic cells (mixed lymphocyte reactions). Suppressor cells from the first culture and responder cells in the second culture were from the same individual. In detail, 3×10^6 normal T cells were incubated in 3 ml culture medium, RPMI 1640 (Flow Laboratories, Inc., Rockville, MD) supplemented with 10% fetal bovine serum (Flow Laboratories, Inc.) with 30 μg Con A (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, NJ) (Con A-activated T cells) or without Con A (nonactivated

control T cells) at 37°C in a humidified 5% CO_2/95% air environment. To both nonactivated control and Con A-activated cultures, 0.2×10^6 mitomycin (Sigma Chemical Co.)-treated monocytes were added. 60 h later, the cells were harvested, washed four times in 0.1 M α-methyl-D-mannoside (Sigma Chemical Co.), treated with mitomycin, and then tested for their suppressor activity in the second assay culture system. For this assay, 1×10^5 mitomycin-treated allogeneic stimulating cells were added to 1×10^5 freshly prepared responder T cells in microtiter plates (Cooke Engineering Co., Alexandria, VA); mitomycin-treated Con A-activated or nonactivated control T cells (1×10^5) from the first culture were then added to measure their ability to suppress the T cell proliferative response to allogeneic cells. 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. These second assay cultures were incubated for 144 h at 37°C in a 5% CO_2/95% air humidified environment. 20 h before the termination of culture period, 1 μCi of [*methyl*-^{3}H]thymidine (5 Ci/mmol; Amersham Corp., Arlington Heights, IL) was added to the cultures and the proliferative response measured as described previously (20, 21, 24, 25).

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 nonactivated T cells − mean counts per minute of unstimulated cultures containing nonactivated T cells)] × 100.

The basic suppressor system was then perturbed by a number of different procedures. In some experiments, normal intact T cells were treated with SLE plasma plus complement before the first culture; in other cases they were treated with SLE plasma plus complement after the first culture (see below).

Treatment of normal T cells with SLE plasma plus complement either before or after Con A activation. 1×10^7 normal T cells before or after activation by Con A for 60 h in 1 ml RPMI 1640 were mixed with 1 ml of SLE plasma, and kept at 4°C for 1 h. These T cells were then washed, resuspended in 750 μl RPMI 1640, and incubated with 250 μl of rabbit complement (preabsorbed with sheep erythrocytes) at room temperature for another 3 h. Thereafter, these cells were washed three times and resuspended in culture medium, after which the viable cell yield was determined by trypan blue exclusion.

Inhibiting effect of sugars on the binding of SLE plasma to desialized T cells. Monosaccharides and polysaccharides were dissolved in RPMI 1640 containing 2% fetal bovine serum to give a concentration of 200 mM. 1×10^6 neuraminidase treated T cells were then floated in 100 μl of the sugar solution plus 100 μl of SLE plasma for 1 h at 4°C. Thereafter, these cells were analysed by indirect immunofluorescence with fluorescein isothiocyanate-conjugated rabbit anti-human IgM with the fluorescence microscope.

RESULTS

Binding of SLE plasma to intact or desialized normal T cells. Table I shows the results of staining of normal T cells, either intact or neuraminidase treated, with normal plasma or SLE plasma, and counterstaining with fluorescein-tagged antibody to human IgM. All plasma from nine patients with active SLE (patients 1–9 in Table I) demonstrated IgM binding to intact normal T cells by the indirect immunofluorescence technique (range 10.7–90.3%). Plasma from SLE patients with either mildly active (patients 10–13) or inactive (patients 14–18) disease and from healthy controls did not contain such antibodies. The results presented here indicate that only plasma from patients with active SLE has IgM antibodies to intact T cells.

TABLE I
*Staining of Either Intact or Desialized Normal T Cells with SLE Plasma Plus Fluorescein-conjugated Antibody to Human IgM**

Plasma source	Cells stained with plasma plus fluorescein-conjugated antihuman IgM	
	Intact T cells	Desialized T cells
	% staining cells	
Active SLE		
1	25.4	84.5
2	16.0	51.9
3	11.8	96.6
4	90.3	92.7
5	19.8	97.1
6	17.4	54.2
7	21.5	71.1
8	10.7	94.0
9	16.6	87.7
mean±SE	25.5±8.2	81.1±5.9
Mildly active SLE‡		
10	0	57.3
11	1.7	75.2
12	1.0	55.8
13	0.7	26.7
mean±SE	0.9±0.4	53.8±10.0
Inactive SLE		
14	0.8	28.8
15	0	18.0
16	1.0	17.9
17	0	30.0
18	0	38.4
mean±SE	0.4±0.2	26.6±3.9
Normal		
mean±SE	0.2±0.2	4.3±1.4

* Peripheral blood T cells were obtained from normal individuals. These intact T cells or neuraminidase treated desialized T cells were incubated for 1 h at 4°C with plasma either from SLE patients or from normal individuals, followed by fluorescein-conjugated antihuman IgM for another 30 min at 4°C, and then staining cells were counted under a fluorescence microscope.

‡ If patients had mild subjective symptoms and had one or two of the criteria of activity as described in Methods, they were included in the mildly active group.

These are consistent with previous observations of our own (20, 21), as well as with those of others (12, 15, 16), that the presence of IgM SLE antibodies to intact T cells correlates well with disease activity.

Treatment of normal T cells by neuraminidase was found to increase markedly the percentage of brightly staining cells with the active plasma (patients 1–9 in Table I). When desialized normal T cells were used as target cells, the average increase in percentage of brightly staining cells was ~56% with these nine active plasma patients. It should be further noted here that plasma from the other patients with less disease activity (patients 10–18), which could not bind to intact T cells, also demonstrated binding of IgM to an appreciable fraction of desialized normal T cells. In contrast, normal plasma did not stain the desialized T cells at all. These results suggest that some, but not all, antigenic determinants on the T cell surface recognized by SLE anti-T cell antibodies could be masked by sialic acid and can be exposed upon removal of sialic acid by neuraminidase.

The increase in the IgM binding to desialized T cells was most likely not due to proteolytic enzymes that could contaminate the preparation of neuraminidase, because prior treatment of the intact T cells with pronase, trypsin, or papain did not enhance the binding of SLE plasma to such treated T cells (Table II). Rather, as shown in Table II, further treatment of neuraminidase treated T cells led to a decrease in the binding of SLE plasma to these cells. Thus, neuraminidase treatment of T cells appears to lead to enhancement of their binding to SLE anti-T cell antibodies by unmasking antigenic determinants by sialic acid. Moreover, the reduction in binding with proteolytic enzymes suggests that such determinants may be composed of asialo-glycoprotein.

Heterogeneity of SLE anti-T cell antibodies in view of their target cell specificities. The possible specificity of anti-T cell antibodies present in active SLE plasma was studied by prior adsorption of the plasma with either intact T cells or desialized T cells (Fig. 1). The ability of active SLE plasma to bind to intact T cells was completely removed by prior adsorption with either intact T cells or neuraminidase treated desialized T cells. The binding of the plasma to desialized T cells was also abolished by prior adsorption with these cells but only slightly with intact T cells. To determine whether the binding activity of SLE plasma against neuraminidase treated T cells could be removed by prior adsorption of the plasma with increasing numbers of intact T cells, a dose-response experiment was performed. 1 ml plasma was adsorbed with increasing numbers of intact normal T cells, and the supernatant plasma after centrifugation was then examined for ability to bind to either intact or desialized T cells. As shown in Fig. 2, adsorption with as few as 1×10^7 intact T cells completely removed the activity of SLE plasma against intact T cells, whereas the activity against neuraminidase treated T cells still remained in the supernatant plasma even after adsorption with 7×10^7 intact T cells. The remaining activity against desialized T cells when preadsorbed with 7×10^7 intact T cells was roughly the same as the remaining activity when preadsorbed with 1×10^7 intact T cells. These results cannot be explained if one assumes that SLE anti-T cell antibodies contain only one type of antibody. Rather, a possible explanation for the results is that SLE anti-T cell antibodies would comprise at least two types of antibodies with different target cell specificities; one type of these can bind to both intact and desialized T cells and another type can bind only to desialized T cells.

TABLE II

Intact T Cells or Neuraminidase Treated T Cells Treated with Proteolytic Enzymes and Stained with SLE Plasma Plus Fluorescein-conjugated Antibody to Human IgM *

		Treatment of target cells with proteolytic enzyme (μg/ml)					
			Pronase		Trypsin		Papain
Source of plasma	Source of target cells	None	50	200	20	200	600
				% cells stained			
Active SLE, patient 7‡	T cells	21.5	8.0	6.5	24.6	19.1	20.0
	Neuraminidase treated T cells	71.1	41.2	12.1	74.2	66.0	66.2
Active SLE, patient 8‡	T cells	10.7	1.0	1.0	11.1	13.9	14.1
	Neuraminidase treated T cells	94.0	67.3	24.2	98.0	90.8	88.1

* Intact T cells, neuraminidase treated T cells or these cells further treated with proteolytic enzymes were incubated with SLE plasma for 1 h at 4°C. Thereafter, these cells were mixed with fluorescein-conjugated antihuman IgM for 30 min at 4°C, and then studied for the binding of IgM to the various T cell populations by the indirect immunofluorescence technique.

‡ For Patients 7 and 8, see Table I.

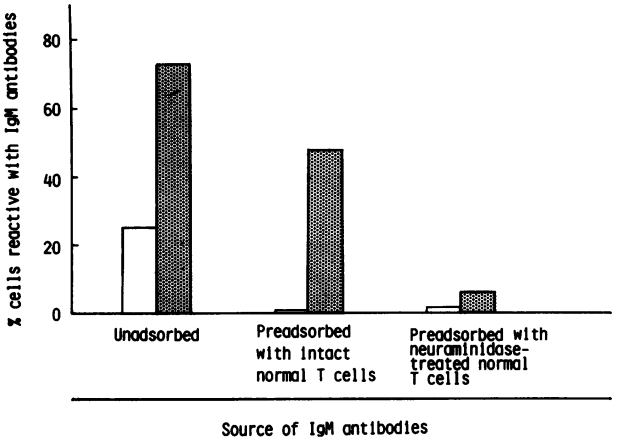

FIGURE 1 Effect of prior adsorption of SLE IgM antibodies with intact normal T cells or neuraminidase treated T cells on the binding of the antibodies to target T cells; a representative experiment. Active SLE plasma was first adsorbed with either intact or neuraminidase treated normal T cells. Either intact or neuraminidase treated normal T cells (target cells) were incubated with such preadsorbed plasma and then further incubated with fluorescein-conjugated antihuman IgM sera. Similar results were obtained in two additional experiments. Target T cells; □, intact normal T cells; ▨, neuraminidase treated normal T cells.

It is unlikely that the observed results can be attributed to nonspecific binding of SLE plasma to desialized cells because active plasma had their reactivity

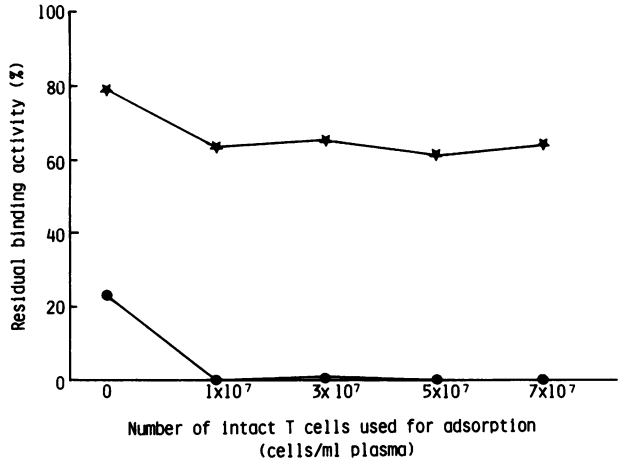

FIGURE 2 Quantitative adsorption analysis of the ability of SLE IgM antibodies to bind to target T cells; a representative experiment. Active SLE plasma was adsorbed with increasing numbers of intact normal T cells. The binding of the preadsorbed plasma to either intact or neuraminidase treated T cells (target cells) was examined by the indirect immunofluorescence. Similar results were obtained in two additional experiments. Target T cells; ●, Intact T cells; °, neuraminidase treated T cells.

to neuraminidase treated T cells removed partially by extensive adsorption with neuraminidase treated non-T cells or neuraminidase treated erythrocytes (Table III). Thus, SLE antibodies directed at desialized T cells appear to be specific, at least in part, for T cells.

Relationship of Con A-induced suppressor T cell activity to the presence of IgM antibodies to desialized T cells. We have previously found that in active SLE patients with defects in suppressor cell generation, most, but not all, have IgM anti-T cell antibodies in their plasma which cause the same defects in a normal T cell population (21). As more and more experience with SLE patients has accumulated, however, it has also become evident that there exist some patients with active disease and some patients with less disease activity who have profound defects in the suppressor cell function but who do not have detectable IgM antibodies to intact T cells in their plasma (Fig. 3A, C). Table IV illustrates two such patients. These patients (Nos. 11 and 12) had mildly active disease, and had defects in the suppressor cell function. Nevertheless, their plasma did not bind to intact T cells from normal donors at all. Antibodies to desialized T cells, however, could be readily detected in their plasma; plasma from patients 11 and 12 bound to 75 and 56% of desialized normal T cells, respectively. Furthermore, plasma from patients 11 or 12 plus complement added to normal intact T cells before Con A activation did not inhibit the generation of functional suppressor cells, whereas treatment of T cells at the end of the Con A activation step markedly blocked the development of suppressor cell function (Table IV). These results suggest that IgM antibodies to desialized, rather than intact, T cells may play a critical role in the elimination of suppressor effector cells in patients with SLE.

11 patients with SLE were studied simultaneously for their Con A-induced suppressor activity and the presence of antibodies to desialized normal T cells in their plasma. In fact, the degree of Con A-induced suppressor function observed in lymphocytes from patients with SLE correlated well inversely ($r = -0.92$) with the presence of IgM antibodies to desialized T cells (Fig. 3B). On the other hand, the presence of IgM antibodies to intact normal T cells did not necessarily correlate with the defects in suppressor function; patients with high titers of IgM antibodies to intact normal T cells had profound defects in the suppressor cell activity. In patients without such antibodies, the suppressor function of their lymphocytes varied greatly among individual patients (Fig. 3A). There was a lesser degree of correlation ($r = -0.58$) between defective suppressor function and the presence of IgM antibodies to intact T cells. We also analysed the relationship of Con A-induced suppressor T cell activity to the pres-

TABLE III

Effect of Prior Adsorption of SLE IgM Antibodies Either with Neuraminidase Treated Non-T Cells or Neuraminidase Treated Erythrocytes on the Binding of the Antibodies to Desialized T Cells

	Source of IgM antibodies						
		Preadsorbed with					
		neuraminidase treated T cells		neuraminidase treated non-T cells		neuraminidase treated erythrocytes	
Experiment	Unadsorbed	3×10^7*	10×10^7*	3×10^7‡	10×10^7‡	1:1§	1:5§
		% desialized T cells stained					
1	96	2	0	70	51	49 (O)‖	44
2	97	0	0	50	46	25 (AB)‖	32

* SLE plasma was incubated with either 3×10^7 or 10×10^7 packed neuraminidase treated T cells/ml plasma at 4°C overnight. The residual capacity of supernatant plasma to bind to neuraminidase treated, desialized T cells obtained from normal individuals was determined by indirect immunofluorescence staining with fluorescein-conjugated anti-human IgM.

‡ Numbers of neuraminidase treated non-T cells used for adsorption (cells per milliliter of plasma).

§ 1 ml SLE plasma were preadsorbed with 1 or 5 ml neuraminidase treated packed erythrocytes.

‖ ABO blood groups used for preadsorption are shown.

ence of IgG antibodies to intact or desialized T cells. The presence of IgG antibodies to either intact or desialized T cells did not correlate with suppressor T cell dysfunction (Fig. 3C, D).

Binding of SLE anti-desialized T cell antibodies to suppressor effector T cells. Our previous studies (2) have shown that the suppressor, but not helper, effector cells reside in the Con A-activated T cell population forming rosettes with autologous erythrocytes, whereas the reverse is true of the Con A-activated T cell population not forming autorosettes. In addition, Con A-activated autorosetting T cells (which exert the suppressive effect) were shown to be stained with fluorescein-labeled peanut agglutinin, which indicates that Con A-induced suppressor effector cells possess abundant β-galactosyl residues exposed on the cell surface, not masked by sialic acid. Additional experiments were performed to determine whether the SLE antibodies to desialized T cells actually bound to suppressor effector cells defined by the autorosette technique. After Con A activation, normal T cells were washed in α-methyl-D-mannoside, treated with SLE anti-T cell antibodies which had been adsorbed extensively with intact T cells (consequently, these antibodies should be specific for desialized T cells), followed by fluorescein isothiocyanate-conjugated $F(ab')_2$ fragment of rabbit antihuman IgM or IgG, and then mixed with autologous erythrocytes to form rosettes. The results of such experiments are shown in Table V. Almost all cells stained with fluorescein-conjugated anti-IgM formed rosettes with autologous erythrocytes; the nonrosetting cells were not stained with the anti-IgM. In contrast, there existed very few cells that were stained with fluorescein-conjugated anti-IgG in the autorosetting T cell population; the nonrosetting cell population was preferably stained with the anti-IgG. These results plus those presented earlier (2) suggest that SLE IgM antibodies to desialized T cells preferentially bind to and kill suppressor effector cells.

Inhibition of reactivity of IgM antibodies to neuraminidase treated T cells by adding various sugars. It has been known that β-galactosyl residues are exposed on cell surfaces after neuraminidase treatment (5, 11, 23). It is possible, therefore, that SLE antibodies to desialized T cells could recognize a terminal β-galactosyl residue of a sugar chain on the cell surface. If this is true, the binding of antibodies to desialized T cells should be inhibited by sugars. Table VI shows that D-lactose profoundly inhibited the binding of SLE plasma to neuraminidase treated T cells. α-D-glucose, D-(+)-mannose, *N*-acetyl-D-glucosamine, and *N*-acetyl-D-galactosamine failed to inhibit the binding of the plasma to desialized T cells. D-(+)-galactosamine and α-D-melibiose inhibited the binding of SLE plasma to desialized T cells, but were not as effective as D-lactose. Thus β-galactosyl residues appear to be an important part of the antigenic determinants for SLE antibodies to desialized T cells.

DISCUSSION

By indirect immunofluorescence, IgM antibodies to desialized T cells were found in all the SLE plasma studied, regardless of the clinical state of the patients;

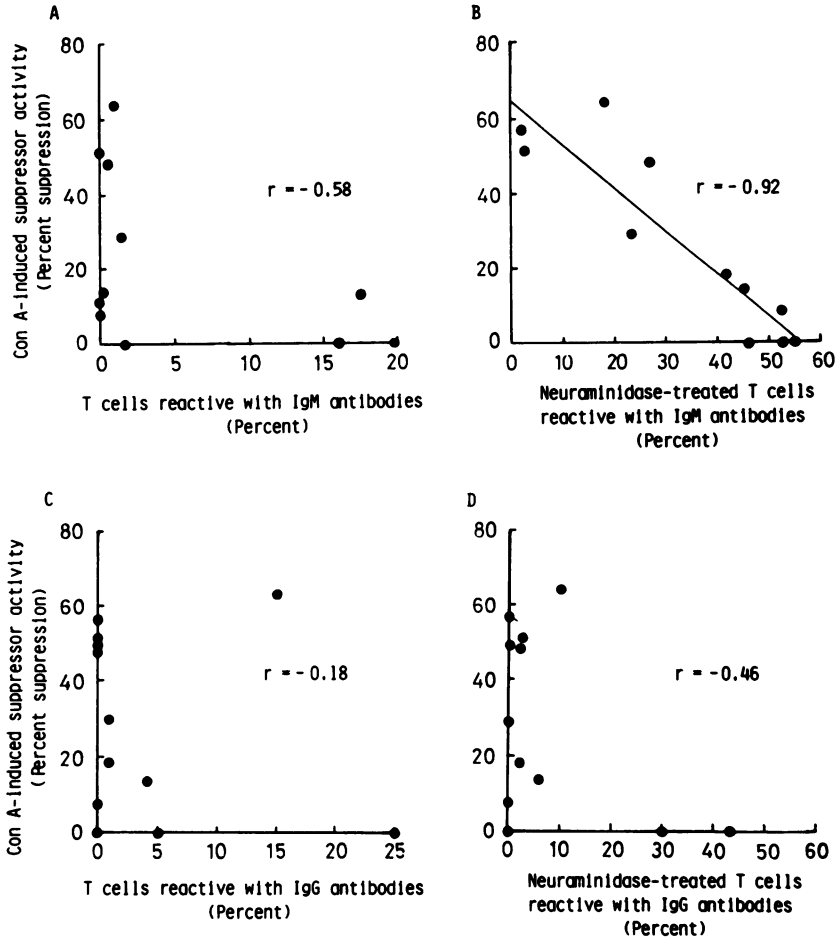

FIGURE 3 Relationship between Con A-induced suppressor T cell activity of lymphocytes from patients with SLE and ability of their plasma to bind to intact or neuraminidase treated T cells. Individual SLE patients were studied simultaneously for their Con A-induced suppressor activity for mixed lymphocyte reactions, and for the presence of IgG or IgM antibodies to intact or desialized T cells by the indirect immunofluorescence.

only plasma from patients with active, but not mildly active or inactive, SLE demonstrated binding of IgM to intact normal T cells. These results suggest that neuraminidase treated, desialized T cells have either more available antigenic determinants that are recognized by SLE antibodies than do intact T cells, or that they have unique antigenic determinants. Prior adsorption of active plasma with intact normal T cells completely removed the ability of such adsorbed plasma to bind to intact T cells, although the considerable activity for neuraminidase treated T cells remained in the adsorbed plasma. Thus, the antibodies are specific for desialized T cells, and recognize unique determinants on such cells. These are not antibodies to desialized cells in general (such as desialized non-T cells or erythrocytes), because prior adsorption of SLE plasma with huge numbers of neuraminidase treated non-T cells or erythrocytes partially removed the activity of the antibodies directed at desialized T cells. These results suggest that the antibodies recognize a unique T cell determinant in association with a sugar determinant.

Antibodies directed at desialized T cells appear to inactivate suppressor effector cells preferentially. Although all active patients with defects in suppressor cell function had antibodies to intact T cells, some mildly active patients, in whom suppressor cell function was lost, had no detectable antibodies to intact T cells; they had, however, high titers of antibodies to desialized T cells. Such SLE antibodies to desialized,

TABLE IV

Lymphocyte Suppressor Function of Particular Patients with SLE and Effect of Their Plasma on Normal Suppressor Effector T Cells

	Con A-induced suppression of mixed lymphocyte reactions
	% suppression
Mildly active SLE, patient 11*	
Lymphocyte suppressor function	11.0
Plasma effect on normal T cells before activation‡	54.6 (49.0)§
Plasma effect on normal T cells after activation‡	12.3 (55.9)§
Mildly active SLE, patient 12*	
Lymphocyte suppressor function	4.4
Plasma effect on normal T cells before activation‡	35.2 (29.3)§
Plasma effect on normal T cells after activation‡	3.9 (31.2)§

* Patients 11 and 12 represent the patients as indicated in Table I. Plasma from patients 11 and 12 bound to 1.7 and 1.0% of intact normal T cells, and to 75.2 and 55.8% of neuraminidase treated normal T cells, respectively.

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

§ Values in parentheses show suppression produced by Con A-activated normal T cells that had received treatment with fetal bovine serum and complement before or after Con A activation in the first culture.

but not intact, T cells were capable of specifically binding to, and inactivating, suppressor effector T cells. In contrast, the very same plasma failed to inactivate suppressor precursor cells in populations of normal T lymphocytes. Thus, our evidence suggests that suppressor effector cells are uniquely susceptible to the effects of antibodies to desialized T cells.

Previous studies (3, 15) have shown that an appreciable fraction of human thymocytes has receptors for peanut agglutinin; in contrast, very few human peripheral blood T cells bind peanut agglutinin unless pretreated with neuraminidase or activated during the course of immune stimulation. These observations suggest that during T cell maturation surface determi-

TABLE V

*Demonstration of Binding of IgM Anti-desialized T Cell Antibodies to Con A-induced Autorosetting T Cells by the Indirect Immunofluorescence**

	Proportion of autorosetting cells stained with			Proportion of nonautorosetting cells stained with		
Source of plasma	IgG SLE antidesialized T cell antibodies	IgM SLE antidesialized T cell antibodies	No staining	IgG SLE antidesialized T cell antibodies	IgM SLE antidesialized T cell antibodies	No staining
			mean %±SE			
Normal (3)‡	5.3±2.9	3.5±2.2	91.5±4.8	5.7±1.8	3.5±2.4	90.8±2.1
Active SLE (7)‡	10.6±2.9	58.0±10.5§	31.4±11.6§	35.1±6.8§	5.7±1.3	59.2±6.3§

* Plasma from patients with active SLE or normal individuals were extensively adsorbed with normal T cells. Normal T cells precultured with Con A were incubated with the adsorbed plasma followed by fluorescein-conjugated antihuman IgG or IgM and then mixed with autologous erythrocytes to form rosettes. The adsorbed plasma could not bind to either intact fresh T cells or T cells precultured without Con A at all.

‡ Number of plasmas tested.

§ Significantly different from the normal group ($P < 0.05$).

TABLE VI
Inhibition of Binding of IgM Antibodies to Neuraminidase Treated T Cells by Various Sugars[*]

Sugars, *100 mM*	Percentage of inhibition
	mean±SE
α-D-glucose	14.3±7.7
D-(+)-galactose	31.6±6.8
D-(+)-mannose	17.3±7.3
N-acetyl-D-glucosamine	5.1±3.0
N-acetyl-D-galactosamine	13.7±10.0
D-lactose	83.4±5.3
α-D-melibiose	43.5±5.1

[*] Four active plasmas were examined.

nants of T cells are masked by sialic acid, but that during the process of activation of suppressor effector cells the sialic acid is again removed. Thus, Con A-activated suppressor effector cells are eliminated by antibodies to desialized T cells (Table IV). Untreated cells from patients with active SLE are markedly enriched in activated T cells (J. J. Smolen, T. M. Chused, A. S. Fauci, and A. D. Steinberg; manuscript in preparation), and such in vivo activated cells are killed by antibodies to desialized T cells. Thus, neuraminidase is not necessary to unmask the determinants necessary for reactivity with the antibodies to desialized T cells, since either Con A activation or in vivo activation is also satisfactory.

Human suppressor effector cells are contained within the autorosetting population (2); these cells possess abundant β-galactosyl residues exposed on the cell surface, not masked by sialic acid (2). We have found that SLE antibodies to desialized T cells selectively bind to, and kill, these suppressor effector cells. Consequently, defects in suppressor function observed in patients with SLE (25–29) could, at least in part, result from antibodies to desialized T cells. Indeed, when patients with SLE were studied simultaneously with regard to (*a*) suppressor cell activity produced by their own T lymphocytes and (*b*) the presence of antibodies to either desialized normal T cells or intact normal T cells, defects in the suppressor T cell activity observed in lymphocytes from patients with SLE correlated well with the presence of antibodies directed at desialized, but not intact, T cells.

It has been shown that β-galactosyl residues are exposed on the cell surface after treatment of cells with neuraminidase (5, 11, 23). Therefore, it is expected that the antigenic determinants recognized by the SLE antibodies to desialized T cells could be associated with β-galactosyl residues. D-(+)-lactose was the most potent inhibitor against the activity of SLE antibodies to desialized T cells, whereas α-D-glucose, D-(+)-mannose, *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine were only slightly inhibitory against the activity. These results suggest that β-galactosyl residues may be an important part of the antigenic determinants recognized by antibodies to desialized T cells. Moreover, it was noted that further treatment of desialized T cells with proteolytic enzymes almost completely inhibited the binding of SLE plasma to these cells. Such an observation strongly suggests that antibodies to desialized T cells may recognize asialo-glycoprotein on the cell surface. Additional characterization and determination of the molecular structure of determinants with which antibodies to T cell subsets interact should lead to further insight into the details of normal immune regulation and defects in such regulation in patients.

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