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Research Article

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Decreased Circulating Thymus-Derived Cells with Receptors for the Fc Portion of Immunoglobulin G in Systemic Lupus Erythematosus

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ABSTRACT Thymus-derived cells with receptors for the Fc portion of immunoglobulin G (Fcγ+ T cells) have recently been found to have a suppressor function, a function that is decreased in systemic lupus erythematosus (SLE). Fcγ+ T cells were found significantly diminished in 21 untreated SLE patients, particularly in the 7 patients who had active disease. Most Fcγ+ T cells were separated with a subpopulation of T cells with low affinity for sheep erythrocytes. Decrease of this subpopulation was dependent on the decrease in Fcγ+ T cells. Non-T cells with Fcγ receptors were also diminished in SLE patients, but their decrease did not correlate with disease activity.

The decrease in suppressor-cell function in SLE may be a result of loss, rather than of dysfunction, of the suppressor Fcγ+ T cells.

INTRODUCTION

A subpopulation of thymus-derived lymphocytes having receptors for the Fc portion of immunoglobulin G (Fcγ+ T cells)[1] is capable of suppressing the differentiation and activation of bone marrow-derived (B) cells (1). This suppressor function of thymus-derived (T) cells is diminished in systemic lupus erythematosus (SLE) (2, 4), as well as in its animal model, the NZB/NZW F_1 hybrid mouse (5). The question of whether this decreased suppression is only functional, or it is because of a decrease in the number of the Fcγ+ T cells that exert it, is particularly important to us because we have found that autoantibodies to cell constituents, such as the antinuclear antibodies found in SLE, may penetrate live mononuclear cells (6), including Fcγ+ T cells, and cause their deletion[2], a process that may take place both in vitro[2] and in vivo (7). We therefore studied the Fcγ+ T cells in the blood of patients with SLE to determine if they are actually decreased. Because Fcγ receptors (FcγR) may be affected by corticosteroids (8), we only studied untreated patients. Lack of treatment was either a result of recent onset or recurrence of active disease, or of inactivation of the disease permitting its withdrawal. We could thus also correlate the number of Fcγ+ T cells to disease activity.

METHODS

Subjects. We studied 21 patients who fulfilled at least four criteria for the classification of SLE (9). None had received corticosteroid or immunosuppressor drugs for at least 3 mo, nor salicylates or nonsteroidal anti-inflammatory drugs for at least 2 wk. 7 had active and 14 had inactive disease, as defined previously (10).

We also studied as controls 21 normal subjects concurrently with the SLE patients.

Cells' separation and identification. We separated mononuclear cells (MNC) in Ficoll-Hypaque gradients (Ficoll, Pharmacia Fine Chemicals, Piscataway, N. J.; Hypaque, Winthrop Laboratories, Sterling Drug, Inc., New York) (11), and from them we separated T cells with low and high affinity for nonneuraminidase-treated sheep erythrocytes (SRBC), and non-T cells on Ficoll-Hypaque gradients under various conditions by the method of West et al. (12, 13) modified as described previously.[2] Briefly, we incubated the MNC 18 h at 4°C with SRBC at a ratio of 70 SRBC per MNC before we layered them on Ficoll-Hypaque and centrifuged them 30 min at 4°C at 1,000 RPM. The pellet contained the rosetted T cells and the interface contained the non-T cells. 3-h incubation of the pellet at 37°C followed by layering and centrifugation on Ficoll-Hypaque allowed separation of the

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[1] *Abbreviations used in this paper*: Fcγ+ T cells, thymus-derived cells with receptors for the Fc portion of immunoglobulin G; FcγR, receptors for the Fc portion of immunoglobulin G; HA-T cells, T cells with high affinity for sheep erythrocytes; LA-T cells, T cells with low affinity for sheep erythrocytes; MNC, mononuclear cells; SLE, systemic lupus erythematosus; SRBC, sheep erythrocytes.

[2] Alarcón-Segovia, D., and A. Ruíz-Argüelles. 1978. Antibody penetration into living cells. II. Anti ribonucleoprotein IgG penetrates into Fcγ+ T cells causing their deletion. Submitted for publication.

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low affinity (LA)-T cells, which detach from the SRBC at this temperature, from the high affinity (HA)-T cells which go to the bottom with the rosetting SRBC. These were lysed with ammonium chloride to free the HA-T cells. The main modification to the method of West et al. (12) was in the use of a third Ficoll-Hypaque gradient to separate the cells with low affinity for SE after incubation at 37°C instead of treating them with ammonium chloride and rosetting them with SRBC. The purpose of this modification was the preservation of the Fc receptor. With this procedure we attained several advantages in the evaluation of Fcγ+ T cells. (*a*) We were certain of recovering the Fcγ+ T cells, most of which have low affinity for SRBC[2] (12, 13). (*b*) We had minimal contamination of our subpopulations of T cells and non-T cells by each other. (*c*) We obtained a T-cell subpopulation enriched in Fcγ+ cells without using their Fc receptor, thereby allowing more accurate counts of Fcγ+ T cells particularly if, as hypothesized, they are diminished in SLE.

We identified cells with receptors for SRBC by erythrocyte rosetting and cells with FcγR by rosetting with antibody-coated chicken erythrocytes as described previously (11). The use of antibody-coated ox erythrocytes instead of antibody-coated chicken erythrocytes did not increase significantly the detection of Fcγ+ cells (data not shown). We identified cells with surface immunoglobulin (Ig) by incubation with polyvalent anti-Ig antibody raised in goats (Behringwerke AG, Marburg, West Germany), and studied the slides under epifluorescent microscopy (6). We identified macrophages by myeloperoxidase staining (14).

Serologic studies. These were done in a blood sample obtained at the same time we drew blood for lymphocyte studies. We determined C_3, C_4, IgG, IgA, and IgM protein levels by radial immunodiffusion in antibody agar plates (Behringwerke, AG); antinuclear antibodies by indirect immunofluorescence on stomach and rat kidney sections, using the same polyvalent anti-Ig antibody; lymphocytotoxic antibodies by a modification of the microcitotoxicity assay of Terasaki and MacClelland (15) on a panel of 10 normal lymphocyte donors, and DNA-binding activity by a modification of the Farr method as described by Leukonia et al. (16).

RESULTS

We confirmed previous findings (11) that there are significantly less circulating Fcγ+ MNC in SLE patients than in normal subjects ($P < 0.0005$), and in untreated SLE patients with active disease than in untreated SLE patients with inactive disease ($P < 0.005$). We also found that the Fcγ+ T cells are significantly diminished in SLE patients as compared to normal subjects, and that this diminution is even more significant in patients with active disease (Table I). All 7 patients with active disease, and 11 of the 14 patients with inactive disease had low absolute numbers of Fcγ+ T cells (less than the mean of normals, 2 SD). The LA-T cells were found significantly diminished in SLE patients in comparison with normal subjects, but were similar in patients with active and inactive disease, except for the diminution ascribable to the more marked lymphopenia found in patients with active disease. The absolute numbers of HA-T cells were also lower in SLE patients, both active and inactive, than in normal controls. Thus, the diminution of total T cells found in SLE depends on both the LA and HA subpopulations.

Most Fcγ+ T cells were separated with the LA-T cell subpopulation where they were markedly diminished in SLE patients as compared to the normal subjects. Patients with active disease had less Fcγ+ T cells in this subpopulation than patients with inactive disease. Dependence of the decrease of LA-T cells on the decrease of the Fcγ+ T cells that are part of them was investigated by linear regression analysis and found to be highly significant ($r = 0.7922$, $P < 0.0005$). Although the HA-T cells contain few cells with FcγR even in

TABLE I

Fcγ+ T Cells in Low- and High-Affinity Subpopulations of T Cells in SLE Patients and Normal Subjects

	T cells*					
	Low affinity		High affinity		Total Fcγ+	
	Total	Fcγ+	Total	Fcγ+		No.
	%		%		%	μl
SLE						
Total (21)‡	24.7±2.2	10.4±1.6	75.4±2.2	0.66±0.3	3.6±0.5	36.5±6.3
Active (7)	22.6±0.9	6.5±1.7	77.4±0.9	0.71±0.3	1.9±0.5	15.6±5.1
Inactive (14)	25.5±3.2	14.5±1.5	74.5±3.2	0.61±0.2	4.3±0.6	47.1±7.9
Controls (21)	39.0±1.5	26.0±1.1	62.3±1.5	3.0±0.4	11.9±0.7	176.2±5.1
Significance						
Total SLE vs. controls, *P*§	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Active SLE vs. controls, *P*¶	<0.0005	<0.0005	<0.0005	<0.005	<0.0005	<0.0005
Inactive SLE vs. controls, *P*¶	<0.0005	<0.0005	<0.0025	<0.0005	<0.0005	<0.0005
Active vs. inactive SLE, *P*¶	N.S.	<0.005	N.S.	N.S.	<0.025	<0.01

* Mean±SEM.
‡ Parentheses indicate number of subjects in each group.
§ Paired Student's *t* test; N.S. = not significant.
¶ Unpaired Student's *t* test.

normal subjects, these were found to be even fewer in SLE patients.

Formation of erythrocyte rosettes was similar in both the LA- and the HA-T cells (92.5±0.4 and 95.8±0.3, respectively). Surface Ig and myeloperoxidase staining was minimal in all subpopulations of T cells obtained in this study (mean 0.14±0.08 and 0.36±0.11, respectively).

We obtained similar numbers of non-T cells in SLE patients and normal controls. However, there were less Fcγ+ non-T cells in SLE patients, active and inactive alike, than in normal controls. These and other characteristics of the non-T cells are shown in Table II.

Although it has previously been shown that the lymphocytophilic antibodies present in SLE do not interfere with T-cell rosette formation (17), we performed 10 experiments with SLE MNC in which we carried out total T-cell rosettes before and after 24-h incubation at 37°C to allow shedding of protein attached to cell surface. These experiments confirmed the lack of interference of SLE sera with T-cell rosettes (mean erythrocyte rosettes±SEM, preincubated cells: 59.2 ±2.0; not-preincubated cells: 58.6±1.9). To further rule out the possibility that immune complexes and(or) lymphocytophilic antibodies present in SLE sera or MNC surface could have caused steric hindrance of receptors for SRBC, thus preventing the separation of Fcγ+ T cells with the rest of the T cells, we did experiments where MNC from four patients with untreated SLE (three active, one inactive) were incubated 24 h at 37°C before the LA-T cell-separation procedure. We also studied normal MNC incubated 2 h at 37°C in undigested as well as pepsin-digested sera from the same active SLE patients. Pepsin digestion was done to prevent antibody penetration through FcγR that might delete Fcγ+ T cells (6). Results of these experiments and their respective controls are summarized in Table III. They allowed us to rule out interference of antibodies and(or) immune complexes present in SLE sera with the detection of Fcγ+ T cells.

Although patients with active SLE had higher DNA binding, higher frequency and titers of antinuclear and lymphocytotoxic antibodies, higher Ig concentration, and lower levels of C_3 and C_4 than patients with inactive disease, linear regression analyses showed no correlation, either direct or inverse, between these serologic findings and the numbers of Fcγ+ T cells.

DISCUSSION

The finding that both T and non-T Fcγ+ circulating cells are diminished in SLE but that only the diminution of Fcγ+ T cells is related to disease activity may indicate that the diminution of the Fcγ+ T cells is important in the pathogenesis of SLE. Its importance could reside in the suppressor function this subpopulation of T cells has (1). Current concepts on the pathogenesis of autoimmunity involve a loss of the suppressor function of T cells on B cells that permits the continuous production of autoantibodies (18). The diminution of Fcγ+ T cells that we have found in SLE is in accord with these concepts. It also indicates that the decrease in suppressor cell function that occurs in SLE may be a result of loss, rather than of dysfunction, of suppressor cells. It is not likely that the diminution of circulating Fcγ+ T cells represents a change in compartmental distribution of Fcγ+ T cells or that it is the result of a conversion of the Fcγ+ T cells to Fcμ cells after their interaction with immune complexes (19),

TABLE II
Characteristics of Non-T Cells Obtained in SLE Patients and Normal Subjects

	Non-T cells*				
	Fcγ+	Surface Ig	Myeloperoxidase staining	Total MNC	No.
	%		%		μl
SLE					
Total (21)‡	22.1±1.3	37.2±1.1	25.9±1.7	33.3±1.6	472.6±34.9
Active (7)	21.5±3.2	37.4±2.5	29.0±2.3	36.0±2.4	423.3±48.7
Inactive (14)	22.3±1.1	37.0±0.8	25.2±2.3	32.5±1.4	497.3±45.9
Controls (21)	29.0±1.5	39.0±1.9	27.1±1.3	32.0±1.7	659.5±30.1
Significance					
Total SLE vs. controls, P§	<0.025	<0.025	N.S.	N.S.	<0.0025
Active SLE vs. controls, P¶	<0.025	N.S.	N.S.	N.S.	<0.005
Inactive SLE vs. controls, P¶	<0.0025	<0.05	N.S.	N.S.	<0.005
Active vs. inactive SLE, P¶	N.S.	N.S.	N.S.	N.S.	N.S.

* Mean±SEM.
‡ Parentheses indicate number of subjects in each group.
§ Paired Student's t test; N.S. = not significant.
¶ Unpaired Student's t test.

TABLE III

Control Experiments to Rule Out Possible Effect of SLE Serum Factors and(or) Membrane-Attached Lymphocytophilic Antibodies or Immune Complexes on the Enumeration of Fcγ+ T Cells

		T cells*			
				Fcγ+	
	MNC	Low affinity	High affinity		No.
	%		%		μl
SLE					
Active (3)‡					
Preincubated§	56.2±2.3	23.1±2.3	76.3±2.3	2.1±0.6	14.7±5.0
Not preincubated	57.5±3.1	22.8±1.7	77.3±1.7	2.4±0.8	16.8±4.8
Inactive (1)					
Preincubated	59	31	69	5.0	50.5
Not preincubated	57	33	67	6.5	65.5
Normal controls (4)					
Preincubated	68.0±1.7	41.1±1.8	58.7±1.8	12.4±2.0	162.3±9.0
Not preincubated	67.1±2.2	40.0±2.2	59.2±2.2	11.7±1.5	153.6±8.2
Preincubated with SLE sera¶	66.8±2.7	42.7±2.0	57.1±2.0	11.1±1.5	144.4±9.2
Preincubated with pepsin-digested SLE sera¶	67.3±2.0	41.5±2.5	58.4±2.5	12.8±2.0	168.3±9.0

* Mean±SEM.
‡ Parentheses indicate number of subjects in each group.
§ 24 h at 37°C.
¶ 2 h at 37°C.

rather than to deletion of these cells. This, however, could not be determined in this study.

The separation of the LA-T cells by using their receptor for SRBC, and the results of experiments where we incubated MNC at 37°C for 24 h to allow shedding of attached proteins, indicate that their diminution could not be ascribed to interference in their detection by immune complexes and(or) lymphocytophilic antibodies. In turn, our finding that the decrease in LA-T cells was primarily a result of a loss of Fcγ+ cells indicates that the circulating Fcγ+ T cells were actually decreased rather than merely blocked.

The loss of Fcγ+ MNC in SLE, that includes loss of Fcγ+ T cells, could be a result of the penetration of antibodies to cellular components into FcγR-bearing cells, a process that we have found to cause loss of these cells in vitro.[2]

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