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Research Article

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A Defect of Immunoregulatory T Cell Subsets in Systemic Lupus Erythematosus Patients Demonstrated with Anti-2H4 Antibody

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Abstract

The cell surface phenotype of peripheral blood lymphocytes (PBL) of systemic lupus erythematosus (SLE) patients was characterized with the anti-2H4 monoclonal antibody that defines the human suppressor inducer subset. The $T4^+2H4^+$ population of cells has been shown to be critical for the activation of $T8^+$ suppressor cells. Patients with SLE had a markedly decreased percentage of $T4^+2H4^+$ cells (13±2%) in their PBL compared with normal controls (21±1%) ($P < 0.001$). This reduction was greatest in patients with active SLE, especially those with renal disease. Serial analysis of patients with SLE and renal disease showed a correlation between percent positive circulating $T4^+2H4^+$ cells and disease activity. Moreover, there was a significant correlation between a low percentage of $T4^+2H4^+$ cells and decreased suppressor–inducer function in autologous mixed lymphocyte reaction–activated $T4^+$ cells from SLE patients. Thus, a deficiency exists in SLE patients with active renal disease in the $T4^+2H4^+$ suppressor–inducer T cell subset.

Introduction

Systemic lupus erythematosus (SLE)[1] is a multisystem disease in which B cell hyperactivity results in the generation of hypergammaglobulinemia and autoantibodies (1–3). Abnormalities of suppressor T cell function have been reported in patients with SLE (4–7); these could be important in the predisposition to or perpetuation of disease activity. Nevertheless, the suppressor cell defect has not been well characterized. Such a defect could result from any of several causes: a numerical or functional deficiency in the suppressor–effector cells or their precursors, a deficiency in inducers of suppressor cells, an increase in helper cell activity, or possible abnormal function of cells that interfere with suppression (contrasuppressor cells).

In earlier studies, when anti-T4 and anti-T8 monoclonal antibodies were initially defined (8–9), we found that SLE patients with multisystem involvement but without renal disease had a high T4/T8 ratio due to a decrease in the number of circulating T8 cells (10). In contrast, patients with SLE manifested by severe renal disease and/or thrombocytopenia had a low ratio of T4/T8 cells (11, 12). Nevertheless, many SLE patients had normal T4/T8 ratios (11, 12). Thus, this ratio alone was insufficient to define a precise defect of the immunoregulatory circuit in these patients.

Previous studies demonstrated that anti–T cell antibodies found in the serum of some patients with active juvenile rheumatoid arthritis (JRA) are reactive with ~ 40% of $T4^+$ cells (13) and that the $T4^+JRA^+$ subset of $T4^+$ cells which reacted with JRA anti–T cell antibodies induces suppressor function. In contrast, the $T4^+JRA^-$ subset functions as the inducer of B cell Ig synthesis in the same system (14). We have recently developed a monoclonal antibody, anti-2H4 (15). This antibody reacted with ~ 40% of $T4^+$ cells but also 50–60% of $T8^+$ cells. In vitro studies indicated that subpopulations of $T4^+$ cells delineated by anti-2H4 were functionally distinct. Whereas $T4^+2H4^+$ cells induced suppressor function, the $T4^+2H4^-$ subset induced helper function in both a pokeweed mitogen (PWM)–stimulated Ig synthesis (15, 16) and an antigen-specific antibody production system (17). Thus, anti-2H4 antibody has similar reactivity to JRA anti–T cell antibodies.

We have now utilized this antibody to characterize peripheral blood lymphocytes from SLE patients. Patients with active SLE had a significantly smaller percentage of circulating $T4^+2H4^+$ suppressor–inducer cells than normal individuals. In fact, patients with active SLE and renal disease had the greatest reduction in the percentage of $T4^+2H4^+$ cells in their peripheral blood lymphocytes (PBL).

Methods

Isolation of lymphocytes. Peripheral blood mononuclear lymphocytes were separated from heparinized venous blood by Ficoll–Hypaque gradient density centrifugation (Pharmacia Fine Chemicals, Piscataway, NJ). The absolute number of peripheral lymphocytes from active SLE patients ($0.1–1.5 \times 10^6$ cells/cm^3) was less than that from inactive SLE patients ($0.5–2.0 \times 10^6$ cells/cm^3) or that from normal controls ($0.8–3.0 \times 10^6$ cells/cm^3). In some experiments, peripheral blood mononuclear cells were further separated into E-rosette positive (E^+) and E-rosette negative (E^-) populations with 5% sheep erythrocytes (Microbiological Associates, Bethesda, MD) as previously described (13, 14). The T cell population obtained was > 94% reactive with a monoclonal antibody, anti-T3, which defines an antigen present on all mature peripheral T cells (8). Furthermore, $T4^+$ cells were isolated by anti-Ig coated plates as described (17). The purity of $T4^+$ cells were > 95%.

Separation of $T4^+$ cells by anti-Ig coated plates. $T4^+$ cells were separated into $T4^+2H4^+$ and $T4^+2H4^-$ subpopulations by anti-Ig coated plates as described (17). In brief, 12×10^6 cells were exposed to 1 ml of anti-2H4 (a 1:125 dilution of ascites) for 30 min at 4°C and then washed to remove excess antibody. 12×10^6 cells suspended in 3 ml media were

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1. *Abbreviations used in this paper:* AMLR, autologous mixed lymphocyte reaction; FITC, fluorescein isothiocyanate; JRA, juvenile rheumatoid arthritis; SLE, systemic lupus erythematosus.

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then applied to a goat anti-mouse Ig antibody-coated plastic plate (Fisher Scientific Co., Pittsburgh, PA). After 70 min of incubation at 4°C, nonadherent and adherent populations were collected. The adherent population is consistently 95% positive with anti-2H4 antibody, and nonadherent cells are consistently 4% positive. These populations are referred to $2H4^+$ and $2H4^-$ cells, respectively.

Patients. The sample population consisted of 69 patients with SLE satisfying the diagnostic criteria of the American Rheumatism Association (18). All patients were monitored at the Arthritis Branch of the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, MD. Disease activity scores were determined by two physicians on a 0-to-4 scale and averaged at the time of blood drawing on the basis of multisystem disease activity including fever, rash, arthritis, serositis, vasculitis, nephritis, and central nervous system disease. Furthermore, the disease activity of patients with renal disease was determined at the time of blood drawing whether the patients had active renal disease and/or activity in extrarenal manifestations. Seven patients were untreated, 35 patients were receiving low-dose corticosteroid therapy (5–20 mg prednisone every other day), 20 patients were receiving intermediate doses of steroids (20 mg prednisone every other day to 35 mg prednisone per day) and seven patients were receiving > 35 mg of prednisone per day. None of the patients had received cytotoxic drugs during the preceding 6 mo at the time of blood drawing. Blood samples were drawn at least 24 h after the last steroid dose. The normal control population consisted of 60 sex- and age-matched healthy individuals who had no significant illness. We judged SLE patients with activity scores ≥ 1 to have active disease and < 1 to have inactive disease. Patients with known renal disease were those with hematuria ($\geq$ 10 RBC/hpf), proteinuria ($\geq$ 1 g/24 h), RBC casts, or cellular casts either currently or in the past. In these patients, lupus renal disease was confirmed by biopsy.

Production of monoclonal antibodies. Three monoclonal antibodies termed anti-T4, anti-T8, and anti-2H4 were used in the present study. Their production and characterization are described elsewhere (8, 14). Anti-T4, anti-T8, and anti-2H4 are available through Coulter Immunology, Hialeah, FL. For the production of the monoclonal antibody anti-2H4, a BALB/c mouse was immunized with cells of a T lymphocyte line derived from PBL of the new world primate *Aotus trivirgatus.* One clone of interest secreted the monoclonal antibody termed anti-2H4. This anti-2H4 antibody reacted with ~ 40–50% of the $T4^+$ lymphocytes and 60% of $T8^+$ lymphocytes. Furthermore, it reacted with non-T cells. In vitro studies indicated that subpopulations of $T4^+$ cells delineated by anti-2H4 were functionally distinct. $T4^+2H4^+$ cells exhibited induction of suppressor function and the $T4^+2H4^-$ subset of lymphocytes showed helper–inducer function in a PWM-stimulated Ig synthesis system (15, 16) and an antigen-specific antibody production system (17).

Analysis of lymphocyte populations with single and two color fluorescence flow cytometry. Single color fluorescence flow cytometric analyses were performed on an Epics V cell sorter (Coulter Electronics, Inc.). Cells were stained with the monoclonal antibodies at a dilution of 1:500, followed by incubation with a $F(ab')_2$ fragment of goat anti-mouse antibody conjugated to fluorescein isothiocyanate (FITC) (Tago Inc., Burlingame, CA). Background fluorescence reactivity was determined with control ascites fluid obtained from mice immunized with a nonsecreting hybridoma. Two color analysis was carried out on an Epics V cell sorter equipped with a dual laser, Argon wavelength 488 nm (FITC) and Krypton wavelength 568 nm (Texas Red), utilizing anti-T4 biotin and anti-2H4 FITC as described before (19). Binding of biotin-conjugated antibodies was detected by incubation with Texas Red (Molecular Probes Inc., Junction City, OR) conjugated to avidin (Calbiochem-Behring Corp., La Jolla, CA) or phycoerythrin–Avidin (Becton-Dickinson & Co., Mountain View, CA) as described previously (19). For each sample, 10,000 cells were analyzed on a log fluorescence scale. Before two-color fluorescence analysis, negative as well as positive samples labeled with a single conjugated antibody or labeled by indirect fluorescence were analyzed. Negative controls for dually stained cells were obtained by staining cell samples with a biotinylated nonimmune mouse IgG antibody and Texas Red and mouse IgG antibody conjugated to FITC. All analyses were performed without knowledge of the patients' clinical status. Data are expressed as percentage of PBL rather than absolute subset cell number because of the known lymphopenia regularly seen in patients with SLE.

Suppressor function of autologous mixed lymphocyte reaction (AMLR)–activated T cells. Suppressor function of T4 cells activated by the AMLR was assessed by addition of AMLR-activated T4 cells to secondary cultures of freshly prepared PBL from a healthy single donor. Briefly, the AMLR was set up in RPMI 1640 supplemented with 10% human AB serum, 200 mM L-glutamine, 25 mM HEPES buffer (Microbiological Associated, Bethesda, MD), 0.5% sodium bicarbonate and 1% penicillin-streptomycin. For a primary culture of AMLR, 2×10^6 responder $T4^+$ cells were cocultured with 2×10^6 irradiated (5,000 Rad) E^- cells in 4 ml total volume of media in 25 cm^2 culture flasks (Falcon Labware, Oxnard, CA). After 7 d, culture cells were layered over Ficoll–Hypaque and centrifuged at 2,000 rpm × 20 min, and then washed three times. AMLR-activated T4 cells (2×10^4) were added to secondary cultures of 1×10^5 freshly isolated PBL from a healthy single donor to determine the suppressor activity of these cells. For PWM-driven IgG synthesis by PBL as a secondary culture, peripheral blood lymphocytes (1×10^5) were cultured in each well of roundbottomed 96-well microculture plates in 200 μl media with 20% heat-inactivated fetal calf serum, in the presence of PWM (Gibco Laboratories, Grand Island, NY) at a final concentration of 1:100 dilution. On day 7, cultures were terminated and IgG in culture supernatants was determined by the solid-phase radioimmunoassay described before (13, 14).

Statistical methods. The Fisher's exact test or the two-tailed unpaired *t* test was used as indicated to calculate *P* values.

Results

PBL expression of T4 and 2H4 in patients with SLE and normal controls are summarized in Table I. The circulating lymphocyte population in normal individuals is composed of 54±2% $2H4^+$ lymphocytes, of which only a portion are $T4^+2H4^+$ suppressor–inducer T cells (21±1% of total). In patients with SLE, the percentage of 2H4 bearing lymphocytes (50±2%) in the peripheral blood was only marginally decreased; however, the percentage of $T4^+2H4^+$ suppressor–inducer cells (13±2%) in the peripheral blood was markedly decreased (Table I). Patients with active SLE had the greatest reduction in percentages of $T4^+2H4^+$ cells (Table I). T4/T8 ratios did not significantly differ between normal controls and SLE patients.

Fig. 1 shows representative two-color profiles of peripheral blood lymphocytes coexpressing the T4 and 2H4 antigens in blood of a normal control, a patient with active SLE and one with inactive SLE. This study was done using anti-T4 biotin with Texas Red avidin and monoclonal anti-2H4 conjugated to

Table I. Cell Surface Characteristics of Lymphocytes of SLE Patients and Normal Controls

Group	Number	Sex ratio	Mean age	% positive subset		T4/T8 ratio
				$2H4^+$	$T4^+2H4^+$	
		F/M	*yr*			
Normals	60	56/4	34 (18–65)	54±2*	21±1	1.7±0.1
Total SLE	69	66/3	34 (21–69)	50±2	13±2§	1.5±0.2
Active SLE	38	36/2	33 (23–58)	46±2‡	11±1§	1.5±0.2
Inactive SLE	31	30/1	34 (21–69)	55±2	16±2‖	1.4±0.2

* Results are expressed as mean±SEM.
‡ $P < 0.01$ as compared with normal controls.
§ $P < 0.001$ as compared with normal controls.
‖ $P < 0.05$ as compared with normal controls.

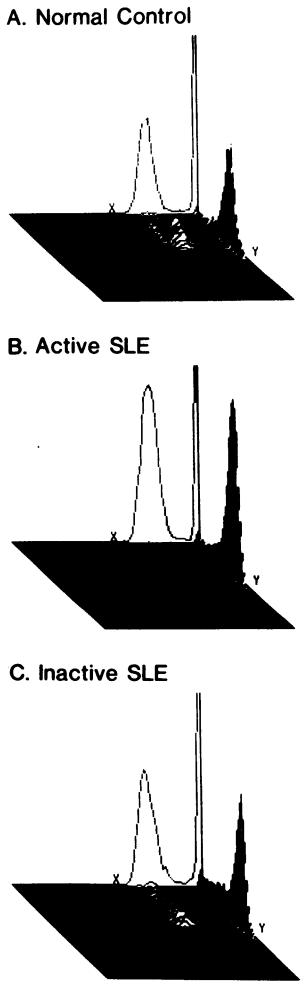

Figure 1. Two-color profiles of peripheral blood lymphocytes coexpressing the T4 and 2H4 antigens in a normal control (*A*), a patient with active SLE (*B*), and a patient with inactive SLE (*C*). Log red fluorescence (the $T4^+$ subset) is shown along *x* axis and log green fluorescence (the $2H4^+$ subset) along *y* axis. Cell number is represented on the vertical axis.

FITC. Log red fluorescence (the $T4^+$ subset) is shown along the *x* axis and log green fluorescence (the $2H4^+$ subset) along the *y* axis. Cell number is represented by the height of the curve. $T4^+2H4^+$ double-positive cells are shown as cell clusters in the center of each panel. In these representative cases, 27% of cells are $T4^+2H4^+$ in the PBL of the normal control, 21.2% of cells are $T4^+2H4^+$ in the inactive SLE patient, and 3.5% of PBL are $T4^+2H4^+$ in the active SLE patient.

The data in Table I suggested a relationship between a reduction in $T4^+2H4^+$ cells and SLE disease activity. The relationship between the percentage of circulating $T4^+2H4^+$ cells in patients and their disease activity was therefore plotted. Fig. 2 shows the relationship between the percentage of $T4^+2H4^+$ cells in PBL and disease activity in the entire group of SLE patients. A lower limit of two standard deviations from the mean percentage of $T4^+2H4^+$ cells in the peripheral blood of normal controls is slightly above 7%. Thus, a patient with 7% $T4^+2H4^+$ cells among their PBL has a significantly lower than normal percentage of circulating $T4^+2H4^+$ cells. There was a significant negative correlation between percent positive $T4^+2H4^+$ cells and disease activity ($\gamma = -0.278$, $P < 0.05$). The relationship between the percentage of circulating $T4^+2H4^+$ cells and disease activity in SLE patients with renal involvement was examined. As shown in Fig. 3, there was a significant negative correlation between percent positive $T4^+2H4^+$ cells and disease activity ($\gamma = -0.375$, $P < 0.05$). Patients with no known renal disease had a lower

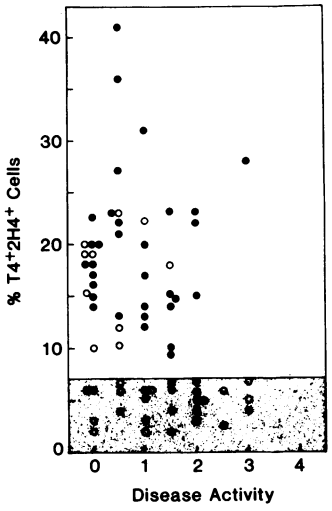

Figure 2. The relationship between the percentage of $T4^+2H4^+$ cells in PBL and disease activity in total SLE patients. Shaded area indicates a lower limit of two standard deviations from the mean percentage of $T4^+2H4^+$ cells in the peripheral blood of normal controls (7%). There was a significant negative correlation between percent positive $T4^+2H4^+$ cells and disease activity ($\gamma = -0.278$, $P < 0.05$). (*Open circles*) SLE patients with known renal disease; (*solid circles*) SLE patients without known renal disease.

negative correlation between disease activity and percent $T4^+2H4^+$ cells ($\gamma = -0.245$, $P > 0.1$).

Table II summarizes the data on the percent positive $T4^+2H4^+$ cells in the PBL of normal controls and SLE patients with different disease types. 13 of 15 (86%) SLE patients with active renal disease had a significant decrease in the percentage of $T4^+2H4^+$ cells in their PBL compared with 7 of 23 (30%) active SLE patients with non-renal disease, 5 of 13 (38%) inactive SLE patients with renal disease, 2 of 18 inactive SLE patients with non-renal disease and 1 of 60 normal controls ($P < 0.0001$, Fisher's exact test).

To determine whether the percent $T4^+2H4^+$ circulating cells of a given patient changed with disease activity, samples were taken from seven patients on at least three occasions. Fig. 4 shows a two-color profile of PBL coexpressing the T4 and 2H4 antigens from an individual with renal disease. As shown in Fig. 4 *A*, there were no detectable $T4^+2H4^+$ cells in the patient's PBL at the time of initial evaluation when the disease was moderately active (activity score 1; $T4^+2H4^+$: 3.1%). As shown in Fig. 4 *B*, $T4^+2H4^+$ cells were detectable 1 mo later at a time when the disease activity was somewhat diminished (activity score 0.5; $T4^+2H4^+$: 8.5%). More importantly, when disease activity was completely inactive 6 mo later, the percent positive $T4^+2H4^+$ cells returned to normal levels (activity score 0; $T4^+2H4^+$: 20%) (Fig. 4 *C*). In this case, the patient did not receive corticosteroids

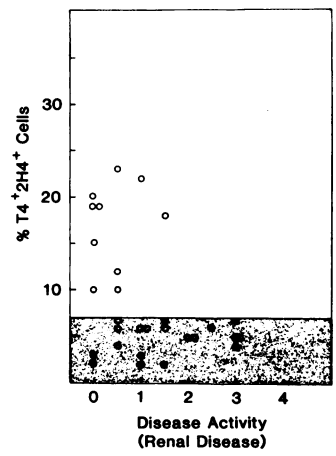

Figure 3. The relationship between the percentage of $T4^+2H4^+$ cells and disease activity in SLE patients with known renal disease. There was a significant negative correlation between percent positive $T4^+2H4^+$ cells and disease activity ($\gamma = -0.375$, $P < 0.05$).

Table II. $T4^+2H4^+$ Cells in SLE Patients with Different Disease Type and Activity

Population	$T4^+2H4^+$ cells (%)		
	Decreased (≤7)*	Within normal range (>7)	Decreased/Total
Patients with active SLE			
Renal disease	13‡	2	20/38§
Non-renal disease	7	16	
Patients with inactive SLE			
Renal disease	5	8	7/31
Non-renal disease	2	16	Total SLE 27/69‖
Healthy controls	1	59	1/60

* Normal range is defined as two standard deviations from the mean percentage of $T4^+2H4^+$ T cells from healthy controls and equaled <7%.

‡ $P < 0.0001$ (Fisher's exact test) compared with active patients with non-renal disease, inactive patients with both renal and non-renal disease and healthy controls.

§ Active SLE, significantly different from inactive SLE ($P < 0.025$).

‖ Total SLE, significantly different from normal controls ($P < 0.001$).

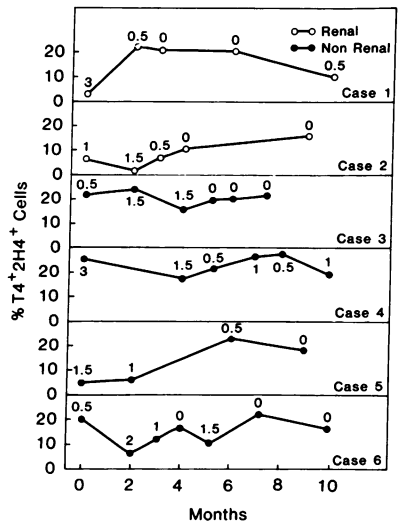

Figure 5. The summary of the percent $T4^+2H4^+$ cells over a period of time in six other patients with changing disease activity. The percentage of $T4^+2H4^+$ cells is shown in the *y* axis over time. Numerals adjacent to the data points represent the SLE disease activity score at that time.

before the initial evaluation of $T4^+2H4^+$ cells and then received a moderate dose of corticosteroids (prednisone, 30–35 mg every other day) until the final evaluation.

Fig. 5 shows a summary of the percent circulating $T4^+2H4^+$ cells over a period of time in 6 other patients with changing disease activity, with or without renal involvement. For the patients with renal disease (cases 1 and 2), decreased percentages of $T4^+2H4^+$ cells correlated with disease activity. In some SLE patients without renal involvement (cases 3 and 4), the percent positive $T4^+2H4^+$ cells did not always correlate with the disease activity over time. Others with nonrenal SLE (cases 5 and 6) did demonstrate a negative association between the percent positive $T4^+2H4^+$ cells and disease activity.

We recently demonstrated that $T4^+$ cells activated in AMLR can function as suppressor–inducer cells in a system involving PWM-driven IgG synthesis (20). Furthermore, the $T4^+2H4^+$ subset of cells proliferated maximally in AMLR and the suppressor–inducer activity of $T4^+$ cells generated in the AMLR response was attributable to an activated $T4^+2H4^+$ suppressor–inducer subset (20). To ascertain these points, $T4^+$, $T4^+2H4^+$, or $T4^+2H4^-$ cells from normal individuals were triggered with autologous non–T cells for 7 d and added to freshly isolated PBL from a healthy single donor to assess their effect on PWM-driven IgG synthesis. As shown in Table III, AMLR-activated $T4^+$ and $T4^+2H4^+$ cells had a strong suppressor activity in a dose-dependent fashion. In contrast, AMLR-activated $T4^+2H4^+$ cells had no such effect. It should be noted that there was no suppression observed when AMLR-activated $T4^+$ cells were added to B+T4 cells with PWM in the absence of $T8^+$ cells, thus indicating that these cells functioned as suppressor inducers (20). Therefore, these results reconfirmed that AMLR-activated $T4^+2H4^+$ cells but not $T4^+2H4^-$ cells showed the suppressor–inducer activity.

Next, to determine whether the decreased percent of $T4^+2H4^+$ suppressor–inducer cells was associated with the defect of generating suppressor activity by AMLR-activated T4 cells, we studied the relationships between percent $T4^+2H4^+$ cells and percent suppression by AMLR-activated T4 cells from SLE patients with known renal disease. To examine suppressor activity, T4 cells from patients with SLE were triggered in AMLR for 7 d and then AMLR-activated T4 cells (2×10^4) were added to 1×10^5 freshly isolated PBL from a healthy single donor to assess their effect on PWM-driven IgG synthesis. As shown in Fig. 6, 15 patients with SLE were studied in this fashion and a significant correlation between a low percentage of $T4^+2H4^+$ cells and a decreased percent suppression of PWM-driven IgG synthesis by

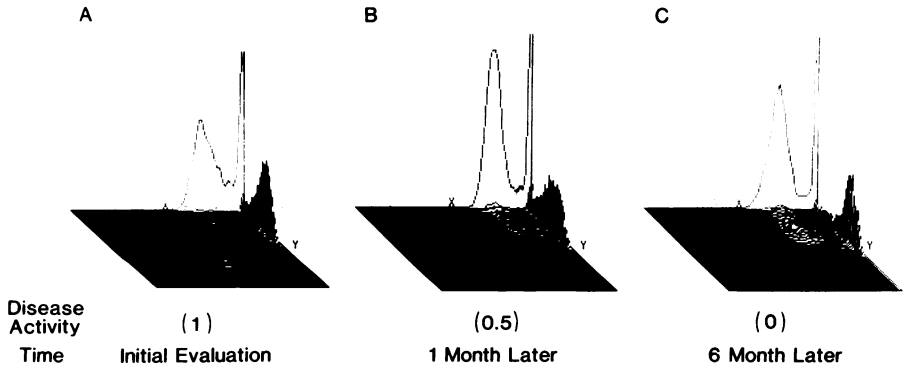

Figure 4. Two-color profile of PBL coexpressing the T4 and 2H4 antigens from a SLE patient with renal disease changed with disease activity. Log red fluorescence (the $T4^+$ subset) is shown along the *x* axis and log green fluorescence (the $2H4^+$ subset) along *y* axis. Cell number is represented on the vertical axis.

Table III. Effect of AMLR-activated Subsets of T4 Cells on PWM-driven IgG Synthesis

Regulator cells*		IgG (ng/ml) Exp. 1	Exp. 2	Exp. 3
—		9200±200‡	8800±600	7600±320
AMLR T4	5×10^3	8300±880 (9)§	8080±480 (8)	6800±960 (11)
	1×10^4	5600±250 (45)	5920±400 (33)	4400±560 (42)
	2×10^4	3640±310 (71)	3200±240 (64)	3640±480 (52)
AMLR $T4^+2H4^+$	5×10^3	4040±460 (56)	2560±320 (71)	2560±240 (66)
	1×10^4	830±60 (91)	1920±400 (78)	960±40 (87)
	2×10^4	550±80 (94)	800±40 (91)	240±40 (97)
AMLR $T4^+2H4^-$	5×10^3	9500±680 (−3)	8160±1240 (7)	7200±560 (5)
	1×10^4	9700±510 (−5)	8960±720 (−2)	7600±480 (0)
	2×10^4	9850±540 (−7)	9600±1600 (−9)	8200±960 (−8)

* Increasing numbers of 7-d AMLR-activated subsets of T4 cells in healthy individuals (5×10^3–2×10^4) were added to freshly isolated PBL from a single healthy donor with PWM to assess their immunoregulatory role. ‡ Results were expressed as mean of triplicate samples±SD. § Parenthesis showed percent suppression calculated as follows: 1 − (observed IgG/IgG in no regulatory cells) × 100.

AMLR-activated T4 cells from SLE patients was found ($\gamma = 0.90$, $P < 0.001$). These results further support the notion that the $T4^+2H4^+$ cells may play an important role in generating suppressor–inducer activity in vivo.

To exclude the possibility that the low percent of $T4^+2H4^+$ cells in patients with active renal disease may be due to the presence of autoantibodies bound to cell membrane antigens interfering with anti-2H4 staining, we selected patients who had anti–T cell antibodies in their sera as previously described (21). Then T cells from normal donors were incubated with these sera for 1 h at 4°C, and after extensive washing these cells were stained with anti-2H4 antibody or anti-T4 antibody and goat anti–mouse Ig FITC. As shown in Table IV, incubation of T cells with SLE sera did not interfere with anti-2H4 and anti-T4 staining of T cells. Thus, these results indicated that the above possibility was unlikely.

Discussion

Anti-2H4 reacts with ~ 40–50% of $T4^+$ lymphocytes and 50–60% of $T8^+$ lymphocytes in man (15). In vitro studies indicated that the $T4^+2H4^+$ subset of lymphocytes exhibited the inducer of suppressor function similar to that previously described as the $T4^+JRA^+$ subset (13, 14) that induces or activates $T8^+$ cells to exert suppressor function. In contrast, the $T4^+2H4^-$ subset of lymphocytes induced helper function in both a PWM-stimulated Ig synthesis (15, 16) and an antigen-specific antibody production system (17). Furthermore, the $T4^+2H4^+$ subset proliferates maximally in response to autologous non–T cells but poorly to soluble antigen stimulation. The $T4^+2H4^-$ subset, in contrast, proliferates relatively poorly to autologous non–T cells but well to soluble antigen stimulation.

The present study utilizes the anti-2H4 monoclonal antibody to characterize the cell surface phenotype of peripheral blood lymphocytes in SLE patients with varying disease activity and in subgroups of patients with and without known renal disease.

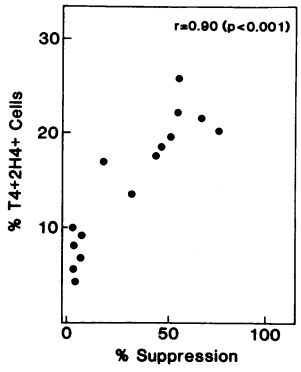

Figure 6. The relationship between the percent of $T4^+2H4^+$ cells and the percent suppression of PWM-driven synthesis by AMLR-activated cells from patients with SLE. Percent suppression was calculated as described in Table III.

Table IV. Anti-T Cell Antibodies in Patients with SLE Did Not Interfere with Anti-2H4 Staining

	Staining with monoclonal antibodies	
	Anti-T4	Anti-2H4
	%	%
Exp. 1		
T cont cells*	64	51
T cells incubated with serum A‡	62	53
Exp. 2		
T cont cells	69	46
T cells incubated with SLE serum B	68	45
Exp. 3		
T cont cells	57	44
T cells incubated with SLE serum C	59	43

* T cont cells were incubated with normal human AB serum for 1 h at 4°C.
‡ T cells were incubated with SLE serum who had anti-T cell antibodies for 1 h at 4°C.

As a group, patients with SLE had a significant reduction in the $T4^+2H4^+$ percent of their PBL. The greatest reduction was in patients with known renal disease and active SLE. There was a significant decrease in the percent $T4^+2H4^+$ cells in 13 of 15 active patients with known renal disease. Because patients with active SLE have a decrease in circulating lymphocytes, the absolute reduction in number of $T4^+2H4^+$ cells is even more marked. It should be noted that the anti-2H4 antibody not only reacts with a part of $T4^+$ cells but also reacts with a part of $T8^+$ cells and non–T cells. Although in the present study we showed that there was the significant decreased percent of $T4^+2H4^+$ cells in SLE patients with active renal disease, decreased percent of $T8^+2H4^+$ cells in such patients was also observed (data not shown). Further studies were now in progress to determine the functional role of $T8^+2H4^+$ cells and 2H4 bearing non–T cells and their significance to autoimmune diseases such as SLE.

In previous studies, SLE patients with multisystem involvement but without renal disease often had a high T4/T8 ratio due to a decrease in the number of $T8^+$ (10–12). In contrast, patients with SLE manifested by severe renal disease and/or thrombocytopenia tended to have a low T4/T8 ratio due to a decreased percent of $T4^+$ inducer cells and increased percent of $T8^+$ cells (11, 12). Nevertheless, many patients with SLE had a normal T4/T8 ratio and shared some clinical features of the other two groups (12). Thus, the ratio itself was insufficient to define a precise defect of the immunoregulatory circuit in most SLE patients. The anti-2H4 antibody adds another parameter to the analysis of human regulatory cells that may prove useful for the characterization of the cellular basis for the defects in patients with autoimmune diseases. Furthermore, the present study shows that there was a significant correlation between a low percent of $T4^+2H4^+$ cells and a decreased percent of PWM-driven IgG synthesis by AMLR-activated $T4^+$ cells from SLE patients.

Because patients with SLE manifest lymphopenia (22–24) and impaired T cell function (25–28), a relationship between the anti–lymphocyte antibodies and this impaired T cell function has long been sought. The precise mechanism of loss of circulating $T4^+2H4^+$ cells in patients with active renal disease is not clear at the present time. We have previously shown, however, that many patients with SLE have anti–T cell antibodies in their sera that react with a functional $T4^+$ suppressor–inducer subset of lymphocytes (21). Thus, sera of the patients with a loss of $T4^+2H4^+$ cells could have anti–T cell antibodies that are reactive with the $T4^+2H4^+$ subset of cells and play some role in their elimination from the circulation.

Although some patients with non–renal disease had a selective decrease in percent of $T4^+2H4^+$ cells, many other patients had normal percentage of $T4^+2H4^+$ cells. The defects in immunoregulatory circuits in these individuals might include abnormal $T8^+$ suppressor cells, B cells, monocytes, or possibly functional abnormalities.

A selective loss of $T4^+2H4^+$ cells is not only limited to patients with active SLE and renal disease. Our recent studies indicate that many patients with progressive multiple sclerosis have a selective decrease in the percentage of circulating $T4^+2H4^+$ cells (C. Morimoto et al., unpublished data). In contrast, there are normal numbers of circulating $T4^+2H4^+$ cells in patients with other neurological diseases. Thus, the present study strengthens the potential importance of the anti-2H4 antibody in studying immunoregulatory defects in autoimmune diseases.

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