Defective Expression of the 2H4 Molecule after Autologous Mixed Lymphocyte Reaction Activation in Systemic Lupus Erythematosus Patients

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Abstract

Previous studies demonstrated that patients with active systemic lupus erythematosus (SLE), especially those with active renal disease, had a marked reduction in $T4^+2H4^+$ suppressor inducer cells in their peripheral blood. However, it was puzzling to find that active SLE patients without renal diseases often had normal percentages of $T4^+2H4^+$ cells. In the present study, we attempted to determine whether active SLE patients bearing normal percentages of $T4^+2H4^+$ cells had a defect in their expression of the 2H4 molecule on $T4^+$ cells after autologous mixed lymphocyte reaction (AMLR) activation. The peripheral blood lymphocytes (PBL) from 50 SLE patients with normal percentages of $T4^+2H4^+$ cells ($\geq$ 7% in PBL) were studied and the results were compared with those of 40 normal individuals. The density of the 2H4 molecule on T4 cells from normal controls increased during the 7-d AMLR; in contrast T4 cells from patients with SLE, especially those with active SLE, had defective expression of the 2H4 antigen after AMLR activation. Patients with inactive SLE, like normals, showed an increase in the 2H4 molecule after AMLR activation. Moreover, a strong correlation was observed between percent suppression of pokeweed mitogen (PWM)-driven IgG synthesis and the density of the 2H4 antigen on AMLR-activated T4 cells. Serial analysis of patients with SLE showed that the density of the 2H4 antigen expression and the suppressor inducer activity of AMLR-activated T4 cells were inversely correlated with disease activity. Thus, defective expression of the 2H4 antigen may be an important mechanism for the failure of active SLE patients with normal percentages of $T4^+2H4^+$ cells to generate suppression.

Introduction

The anti-2H4 antibody subdivides the total CD4 (T4) population into suppressor inducer and helper inducer subsets (1). The $T4^+2H4^+$ subset of cells exhibits suppressor inducer function by activating $T8^+$ cells to exert suppressor effector activity; the $T4^+2H4^-$ subset of cells exhibit helper function in both pokeweed mitogen (PWM)-stimulated IgG synthesis and antigen-specific antibody production system (2, 3). In the autologous mixed lymphocyte reaction (AMLR)[1] the $T4^+2H4^+$ subset of cells proliferated maximally, and moreover, the suppressor activity of $T4^+$ cells generated in this response was attributable to an activated $T4^+2H4^+$ suppressor inducer cell (3). In this system, anti-2H4 antibody uniquely blocks the suppressor inducer function of AMLR-activated $T4^+2H4^+$ cells, suggesting that the 2H4 molecule itself (a 200/220 kD glycoprotein) (3, 4) may be involved in suppressor inducer function (3).

Systemic lupus erythematosus (SLE) is a multisystem disease characterized by production of a variety of autoantibodies, many of which are reactive with nuclear antigens and determinants on the surface of leukocytes (5–9). In earlier studies, we have shown that patients with SLE had a markedly decreased percentage of $T4^+2H4^+$ cells among their peripheral blood lymphocytes (PBL) (10). This reduction was greatest in patients with active SLE, especially those with renal disease. Furthermore, serial analysis of patients with SLE and renal disease showed an inverse correlation between the percentage of circulating $T4^+2H4^+$ cells and disease activity. Patients with active SLE but no renal involvement had normal percentages of $T4^+2H4^+$ cells.

Given the apparent importance of the 2H4 molecule in generating suppressor signals in the AMLR system, we sought to determine whether those SLE patients with normal percentages of $T4^+2H4^+$ cells might have a defect in 2H4 antigen expression after AMLR activation. In earlier studies we found that the density of the 2H4 molecule on normals' $T4^+$ cells increased during the 7-d period after AMLR activation. In the studies reported below, we show that in patients with active SLE, there was defective expression of the 2H4 antigen on $T4^+$ cells after AMLR activation. On the other hand, inactive SLE patients, like normals, showed an increase in density of the 2H4 molecule during AMLR activation. Defective expression of the 2H4 antigen in patients with active SLE correlated with a decrease in the generation of suppression. Taken together, these results suggest that, in patients with active SLE and normal percentages of $T4^+2H4^+$ cells, a defect in 2H4 antigen expression may be an important mechanism for their impairment of capacity to generate suppression.

Methods

Isolation of lymphocytes. Peripheral blood mononuclear lymphocytes from normal individuals and SLE patients were separated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation (Pharmacia Fine Chemicals, Piscataway, NJ). Peripheral blood mononuclear cells were further separated into E rosette–positive (E^+) and E rosette–negative (E^-) populations with 5% sheep erythrocytes

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1. *Abbreviations used in this paper:* AMLR, autologous mixed lymphocyte reaction; LCA, leukocyte common antigen; MCN, mean channel number; PE, phycoerythrin; RBC, red blood cell; SLE, systemic lupus erythematosus.

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(Microbiological Associates, Bethesda, MD) as previously described (1–4). 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 (11). Furthermore, $T4^+$ cells were isolated by anti-Ig–coated plates as described (2). The purity of $T4^+$ cells was > 95%.

Patients. The sample population consisted of 50 patients with SLE and normal numbers of $T4^+2H4^+$ cells (≥ 7% in PBL), satisfying the diagnostic criteria of the American Rheumatism Association (12). All patients were monitored at the Arthritis Branch of the National Institute of Health, Bethesda, MD. Five patients were untreated; 45 patients were receiving low to intermediate doses of prednisone (5 to 25 mg/d). None of the patients had received cytotoxic drugs during the preceeding 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 40 sex- and age-matched healthy individuals who had no significant illness. Patients with known renal disease were those with hematuria (≥ 10 red blood cells per [RBC/high-powered field]), proteinuria (≥ 1 g per 24 h), RBC casts, or cellular casts, either currently or in the past. In these patients, lupus renal disease was confirmed by biopsy.

Disease activity score. Disease activity scores were determined for each patient at the time of blood drawing by two physicians on the basis of multisystem disease manifestations. Each grader provided a score on a semiquantitative zero to four scale with regard to global lupus disease activity. Manifestations included fever, rash, arthritis, serositis, vasculitis, active nephritis, and active central nervous system disease. The severity of generalized symptomatology, the degree of active disease in each organ system, and the number of organ systems involved with active disease all were assessed to arrive at a score. This grading scheme is a condensation of a zero to ten scale used previously (13). The scores from the two graders were averaged. Patients with scores of two or higher were considered to have substantially active disease; those with scores of one or one and one-half had milder active disease; and patients scores under one were deemed to be inactive.

Monoclonal antibodies. Five monoclonal antibodies termed anti-CD4, anti-CD8, anti-2H4, anti-IL2R and anti-Ia were used in the present study. Their production and characterization are described elsewhere (1, 11, 14, 15). All monoclonal antibodies described above are available through Coulter Immunology, Hialeah, FL.

Analysis of lymphocyte populations with single- and two-color fluorescence flow cytometry. Single- and two-color fluorescence flow cytometric analyses were performed on an Epics V cell sorter (Coulter Electronics, Inc.). For single-color analysis, 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 staining was performed using anti-T4 FITC and anti-2H4 phycoerythrin (PE) (Coulter Immunology) as described before (10). For each sample, 10^4 cells were analyzed on a log fluorescence scale. Negative controls for dually stained cells were obtained by staining cell samples with nonimmune mouse IgG antibody separately conjugated to PE and FITC. Flow cytometric standardization was achieved by running a fluorescent 4.05-μm bead, which exhibited constant light scatter and fluorescence properties. The flow cytometer was run on a fixed laser power (600 mW at 488 nm) over the duration of the study. Control and patients samples were run with the PE photomultiplier adjusted to a high enough voltage such that the fluorescence histogram derived from the forward angle vs. side scatter–gated lymphocyte population yielded 2–7% background autofluorescence intensity in the range reserved for positively PE-stained cells. All analyses were performed without knowledge of the patient's clinical status.

Culture conditions. AMLR cultures were prepared in RPMI 1640 supplemented with 10% human AB serum, 4 mM L-glutamine, 25 mM Hepes buffer (Microbiological Associates), 0.5% sodium bicarbonate, and 1% penicillin-streptomycin. For the primary AMLR culture, 2.5×10^6 responder T4 cells were cocultured with 2.5×10^6 irradiated (5,000 rad) E^- cells in 5-ml total volume of media in 25-cm^2 culture flasks (Falcon Labware, Oxnard, CA) in 10% AB serum media as described above. At the same time, T4 cells without irradiated E^- cells were also prepared in 25-cm^2 culture flasks as a control culture. After 7 d, culture cells were layered over Ficoll-Hypaque, centrifuged at 2,000 rpm for 20 min, then washed three times with 2.5% fetal calf serum containing minimum essential medium. For assessing suppressor inducer function, 2×10^4 AMLR-activated T4 cells thus obtained were added to secondary cultures of 10^5 freshly isolated PBL from a healthy single donor in the presence of PWM (1:100 dilution) (Gibco Laboratories, Grand Island, NY) and cultured in each well of round-bottomed 96-well microculture plates in 200 μl of RPMI 1640 media with 20% heat-inactivated FCS for 7 d as previously described (3). Production of IgG was detected in culture supernatants by RIA, and the percent suppression by AMLR-activated cells of PWM-driven IgG synthesis of autologous PBL was calculated as percent suppression = (1 − IgG with regulator cells/IgG without regulator cells) × 100.

Results

Patient characteristics. In the present study, patients with SLE and normal percentages of $T4^+2H4^+$ cells (≥ 7% in PBL) were selected. The age, sex, disease type, dose of prednisone, lymphocyte counts, and T4/T8 ratio of SLE patients and normal controls studied are summarized in Table I. Only 4 of the 50 patients with SLE studied had renal involvement. This is consistent with an earlier study which showed that SLE patients with active renal diseases, but not those without renal diseases, had a marked reduction in $T4^+2H4^+$ cells (10). SLE patients were subdivided into three groups by disease activity; active

Table I. Characteristics of Normal Controls and SLE Patients Studied

Parameters	Normal controls	Total SLE patients	SLE		
			Active	Mild active	Inactive
Numbers	40	50	8	27	15
Age	37 (19–63)	36 (17–56)	38 (22–40)	34 (17–51)	38 (24–56)
Sex (F/M)	36/4	45/5	7/1	25/2	13/2
Renal/nonrenal	—	4/46	1/7	3/24	0/15
Prednisone (*mg/d*)	—	10.1±7.7 (0–25)	15.0±3.3 (0–25)	9.9±6.3 (0–22.5)	5.3±8.3 (0–25)
Lymphocytes (*per centimeter*)	1,876±644	1,592±555	1,624±479	1,560±565	1,615±594
T4/T8	1.6±0.1	1.6±0.2	1.6±0.2	1.6±0.2	1.6±0.2

Results are expressed as mean±SEM.

(activity score of two or more), mildly active (activity score of one or one and one-half), inactive (activity score less than one). As shown in Table I, the absolute lymphocyte counts and the T4/T8 ratios were not significantly different among the three groups.

Change in density of 2H4 expression. To determine whether the density of 2H4 antigen expression on AMLR-activated T4 cells from patients with active SLE differs from that of normals, we examined cell-surface 2H4 antigen expression on $T4^+$ cells after AMLR activation. Fig. 1 illustrates 2H4 antigen expression on unstimulated and AMLR-activated T4 cells from a normal control and patients with active, mildly active, and inactive SLE. In normal individuals, the density of the 2H4 antigen expression increased 7 d after AMLR activation, whereas T4 cells cultured with media alone do not demonstrate an increase in 2H4 antigen density. In contrast, the density of 2H4 antigen expression decreased after 7-d AMLR activation in patients with active SLE. Patients with inactive SLE behaved in a manner similar to normal controls, whereas in patients with mildly active SLE, the density of 2H4 antigen expression was virtually unchanged after AMLR activation. The density of the T3 and T4 antigens changed only minimally during the AMLR. Ia and interleukin 2 (IL-2) receptor expression on AMLR-activated cells showed only minimal differences between patient groups and normal controls. Moreover, the number of $2H4^+$ cells did not change appreciably after AMLR activation in normal controls and patients with mildly active and inactive SLE. However, in patients with active SLE, the number of $2H4^+$ cells appears to decrease after AMLR activation.

Fig. 2 shows the cell surface expression of the 2H4 antigen on T4 cells in all individuals studied after stimulation with or without autologous non-T cells. In 40 normal controls, the 2H4 antigen was expressed on 49±8% (mean±SD) of T4 control cells and 50±9% (mean±SD) of AMLR-activated T4 cells, while in patients with active SLE, the percentage of $2H4^+$ cells decreased from 44±7 to 30±8% after AMLR activation ($P < 0.005$). In patients with mildly active or inactive SLE, the percentage of $2H4^+$ cells was little changed after AMLR activation.

The density of 2H4 antigen expression was also examined after AMLR activation. As shown in Fig. 3, the mean channel

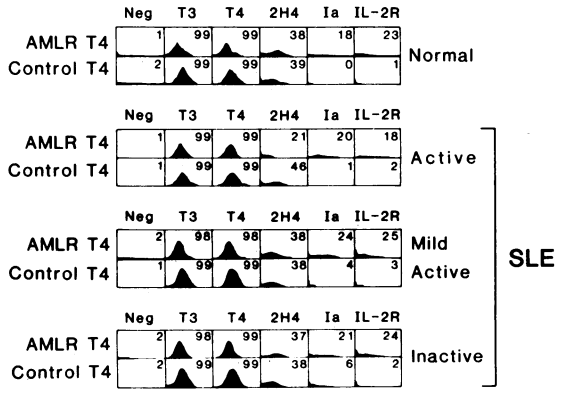

Figure 1. Cell surface antigen expression on control T4 and AMLR-activated T4 cells from a normal control, a patient with active, one with mildly active, and one with inactive SLE. Each histogram shows the number of cells (ordinate) vs. fluorescence intensity (abscissa).

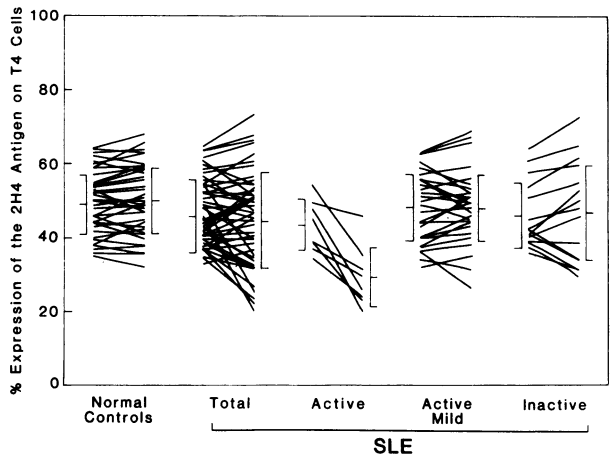

Figure 2. Percentage of 2H4 antigen on T4 cells in all individuals studied. The lines represent the results of studies of cells cultured for 7 d without non-T cells (*left*) compared with the results of cells cultured with autologous non-T cells (*right*). Error bars show mean±standard deviation (SD).

number (MCN) for normal controls ($n = 40$), the 2H4 antigen fluorescence intensity increased from 63±11 to 89±13 (mean±SD) after AMLR activation ($p < 0.005$). In SLE patients overall, a slight increase in the MCN of the 2H4 antigen was observed after AMLR activation, MCN 69±15 (preactivation) vs. MCN 76±17 (postactivation). When SLE patients were divided into three groups by level of disease activity (using activity scores) distinct differences were observed in $2H4^+$ MCN between control T4 and AMLR-activated T4 cells. In patients with active SLE ($n = 8$), 2H4 MCN was decreased from 70±7 to 58±8 after 7 d of AMLR activation ($P < 0.025$). In contrast, in patients with inactive SLE ($n = 15$), the MCN increased from 66±16 to 90±12 after the AMLR ($P < 0.005$), whereas only a slight increase in MCN was noted in mildly active SLE patients ($n = 27$) (MCN 70±16 vs. 73±15)

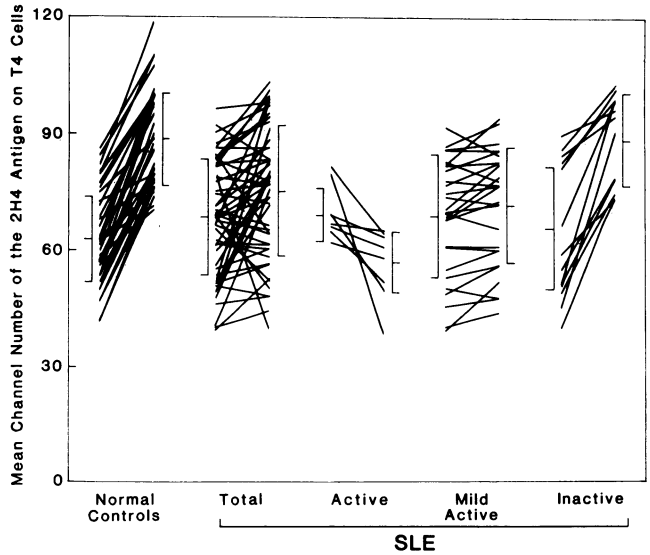

Figure 3. Changes in 2H4 antigen density on T4 cells in all individuals studied, the lines compared results obtained from cells cultured without (*left*) or with (*right*) autologous non-T cells. Error bars show mean±SD.

($p < 0.1$). The above results indicate that, in patients with active SLE, both percent positive cells and the density of 2H4 antigen expression decreased after AMLR activation, compared with control T4 cells.

Studies of suppression of IgG production. Since earlier studies suggested that the 2H4 molecule itself was involved in generating suppressor inducer signals (3, 16), we next determined whether the diminished expression of the 2H4 antigen on AMLR-activated T4 cells in patients with SLE was also associated with a defect in suppressor inducer function. If we add 2×10^4 AMLR-activated T4 cells from normal individuals ($n = 9$) to 10^5 PBL in the presence of PWM, a suppression of immunoglobulin synthesis is observed (57±9%) (Table II). When a similar experiment is carried out with AMLR-activated T4 cells from patients with active SLE, no suppression is observed. Similarly, weak suppression (12±16%) was observed with cells from patients with mildly active SLE (Table II). AMLR-activated cells in patients with inactive SLE, however, generated normal levels of suppression. Thus, the ability to generate suppression appeared to correlate with the density of 2H4 antigen on AMLR-activated T4 cells.

These findings also correlated with proliferative response in the AMLR of patients with SLE. As previously reported (17, 18), we also found a decreased AMLR response in patients with active SLE, a slightly decreased AMLR response in patients with mildly active SLE, and a normal AMLR response in patients with inactive SLE, compared with that of normal controls (data not shown).

Kinetic studies. In a normal control, the 2H4 antigen density was maximum on day 7 (Fig. 4). Suppressor inducer activity of such cells was also shown to increase gradually, with maximal suppression on day 7 and a marked reduction of this activity by day 10. Thus, there was a direct correlation between the capacity to induce suppression and 2H4 antigen density. In a patient with active SLE, however, 2H4 density gradually decreased with time, and almost no suppressor inducer function was observed (Fig. 4). The density of 2H4 on T4 cells cultured in medium alone was virtually unchanged both in normals and patients with SLE.

To further determine whether the defective 2H4 expression on AMLR-activated T4 cells in patients with active SLE may be due to the fact that the $T4^+2H4^+$ cells from those patients may have already been activated in vivo, the AMLR-activated T4 cells (day 7) from a normal donor were restimulated with irradiated autologous non-T cells, and the 2H4 expression on restimulated T4 cells were examined. As shown in Table III, the 2H4 antigen density (mean channel number) on day 7 AMLR-activated T4 cells decreased more rapidly after restimulation of these cells with irradiated autologous non-T cells than did 2H4 on day 7 of AMLR-activated T4 cells cultured without restimulation. Although the above results suggest that T4 cells have a more rapid decrease in 2H4 antigen expression after restimulation, it is still uncertain whether this is the case in the patients with active SLE, since the $T4^+2H4^+$ cells freshly isolated from the peripheral blood in patients with SLE did not express activation antigens such as Ia and IL-2 receptor antigen, which was in contrast to the in vitro–activated $T4^+2H4^+$ cells.

Sequential analysis. To determine whether the defect in 2H4 antigen expression on AMLR-activated T4 cells of a given patient changed with disease activity, samples were taken from five patients on at least three occasions. In addition, we examined the suppressor inducer activity of such cells simultaneously (Table IV). When initially examined, case 1 was clinically inactive. Following activation in AMLR, 2H4 antigen density on T4 cells increased from an MCN of 52 to 76, and the cells themselves showed marked suppressor inducer activity (58% suppression). Subsequently, when the patient became severely ill 3 mo later (activity score of three), we noted a fall in 2H4 antigen expression and density (from 49 to 26% $2H4^+$; MCN from 66 to 60) and impaired suppressor inducer activity (16% suppression). Seven months later, the patient was once again clinically stable. At this time, the MCN of 2H4 antigen expression again increased from 51 to 69, and the suppressor inducer activity increased (43% suppression). The data from four additional patients also showed a similar relationship between disease activity and the density of 2H4 antigen expression and the suppressor inducer activity.

Relationship between prednisone dose and the 2H4 expression. To determine whether steroid treatment may effect the expression of the 2H4 molecule on AMLR-activated T4 cells, we examined the relationships between the dose of prednisone and the ratio of MCN of 2H4 on AMLR-activated T4 cells to that on control T4 cells from patients with inactive SLE. As shown in Fig. 5, there is no correlation between the dose of prednisone and the expression of the 2H4 molecule

Table II. Suppressor Inducer Activity of AMLR-activated T4 Cells from Normal Controls and Patients with SLE with Different Disease Activity

Patient groups	Number	Suppressor inducer activity (percent suppression)	MCN of the 2H4 antigen		$2H4^+$	
			Control	AMLR	Control	AMLR
		%			%	
A. Normal Controls	9	57±9	65±7	91±4	49±8	50±9
B. Total SLE	26	16±29	69±15	76±17	46±10	45±13
Active	6	−14±21	69±6	56±6	44±7	30±8
Mild	14	12±16	68±8	71±11	49±9	49±8
Inactive	6	51±15	65±9	91±7	47±9	48±13

7-d AMLR activated T4 cells (2×10^4) from normal controls and SLE patients were added to freshly isolated PBL from a single healthy donor with PWM to assess their immunoregulatory role. The mean channel number of 2H4 staining is given as the percentage of cells positive for 2H4. In both cases, only T4 cells were studied. Results were expressed as mean±SD.

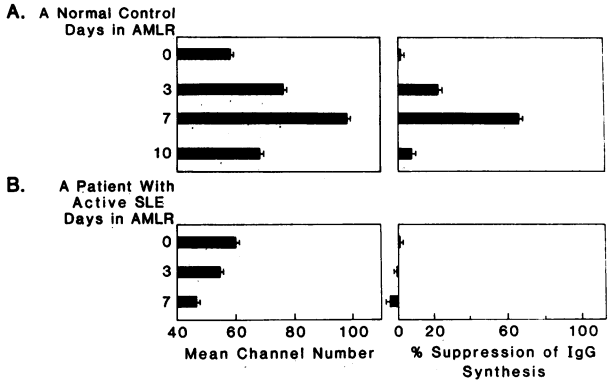

Figure 4. Correlation between density of 2H4 antigen expression and suppressor inducer activity of T4 cells during AMLR in a normal control and a patient with active SLE. T4 cells were cocultured with an equal number of irradiated (5,000 rad) autologous non-T cells for the indicated day. The 2H4 antigen expression (*left*) and suppressor inducer function (*right*) of T4 cells were examined at the same time during AMLR. The abscissa in the left panel shows the mean channel number of 2H4 antigen expression on a logarithmic scale, and the abscissa in the right panel shows percent suppression.

($\gamma = -0.109$; $P > 0.1$). These results suggest that the steroid treatment may not effect the expression of the 2H4 molecule on AMLR-activated T4 cells in SLE patients.

Discussion

Previous studies demonstrated that patients with active SLE had significantly decreased percentages of circulating $T4^+2H4^+$ suppressor inducer cells compared with normal individuals (10). Patients with active SLE and renal disease had the most marked reduction in circulating $T4^+2H4^+$ cells. Although some patients with nonrenal disease had a selective decrease in $T4^+2H4^+$ cells, most had normal percentages of $T4^+2H4^+$ cells, despite active disease (10). In the present study, we attempted to determine whether active SLE patients with normal $T4^+2H4^+$ percentages had impaired 2H4 antigen expression after AMLR activation.

We show here that the density of the 2H4 molecule on T4 cells from normal individuals, as well as inactive SLE patients, increased after AMLR activation compared with that on T4 cells cultured with media alone. In contrast, the density of the 2H4 molecule on T4 cells from patients with active SLE patients decreased after the AMLR. Furthermore, the failure to increase expression of the 2H4 molecule correlated with a decrease in the generation of suppressor function.

A 200/220 kD surface glycoprotein identified by anti-2H4 antibody has previously been shown to be expressed on the suppressor inducer subset ($T4^+2H4^+$), but not the helper inducer subset of T4 cells ($T4^+2H4^-$) (1–3, 19). Moreover, biochemical analysis of the antigens defined by the anti-2H4 antibody has shown that the antibody was specific for the 200/220 kD isoforms of the leukocyte common antigens (LCA) (19, 20). The $T4^+2H4^+$ cells fail to proliferate in response to soluble antigen, but proliferate maximally in the AMLR; the suppressor activity of T4 cells generated in the AMLR response can be attributed to a preferential activation of the $T4^+2H4^+$ suppressor/inducer subset (3). The density of the 2H4 molecule expressed on T4 cells increased during AMLR activation and was closely correlated with the generated suppressor inducer activity of such cells (16), suggesting that the 2H4 molecule might be critical to the suppressor inducer function. This idea is supported by the findings that anti-2H4 antibody blocks the generated suppressor inducer function of AMLR-activated T4 cells (3, 16) and that concanavalin A–induced suppressor T cells, which are $2H4^+$, can be blocked with anti-2H4 antibody (4). The structure of the human LCAs, including 2H4 antigen, deduced from cDNA sequences suggests that the LCAs have very long extracellular and cytoplasmic domains (21). This architecture is commonly found among growth factor receptors that have both receptor activity and intracellular signaling activity (22). Thus the 2H4 molecule may function as either a cell surface receptor for a still unknown ligand or as a cell interaction molecule or both.

Although defective proliferation in the AMLR has been observed in patients with SLE, especially in active stages (17, 18, 23), it is unlikely that the defective expression of the 2H4 antigen on T4 cells in the AMLR is simply due to impaired proliferation. Expression of other cell surface molecules such as T3 and T4 was virtually unchanged. The same was true of Ia antigens and IL-2 receptors on T4 cells, even in active SLE. Additional experiments revealed that although restoration of the defective AMLR proliferation in active SLE was observed in the presence of recombinant IL-2, the defective expression of the 2H4 antigen on AMLR T4 cells was not restored (data not shown). Actually, the defective expression of 2H4 antigen on AMLR T4 cells from such patients became more exaggerated in the presence of IL-2.

Since the average doses of corticosteroid given to patients with active SLE were higher than in patients with inactive SLE, it is possible that steroid treatment may affect the expression of the 2H4 molecule on AMLR-activated T4 cells. However, one patient with active SLE had not received any steroids but still showed defective expression of the 2H4 molecule on T4 cells after AMLR activation. Moreover, the study of the relationships between the dose of prednisone and the ratio of MCN of 2H4 on AMLR-activated T4 cells to that on control T4 cells from patients with inactive SLE indicated that there was no correlation between the dose of prednisone and the expression of the 2H4 molecule (Fig. 5). Furthermore, in the serial study of case 1 (Table IV), the patient had received 22.5 mg/d of corticosteroid from May to August 15, and then had received increased doses of corticosteroid with the increase

Table III. Changes in MCN of the 2H4 Antigen on AMLR-activated T4 Cells Restimulated With or Without Irradiated Autologous E^- Cells

Cell group	MCN of 2H4*	MCN of 2H4‡
Day 7 AMLR-activated T4 cells	138	138
Day after restimulation§		
Day 1	132	122
2	126	108
3	120	98

* AMLR-activated T4 cells without restimulation.
‡ AMLR-activated T4 cells with restimulation.
§ AMLR-activated T4 cells (day 7) were restimulated with or without equal number of irradiated autologous E^- cells for indicated days.

Table IV. Relationship between Suppressor Inducer Function and Expression of the 2H4 Antigen over Time in SLE Patients with Changing Disease Activity

Patient	Date of study	Disease activity	MCN of 2H4 antigen		2H4$^+$ cells		Suppressor inducer function (percent suppression)
			Control T4	AMLR T4	Control T4	AMLR T4	
					%		
1	5/14/85	0	52	76	32	35	58
	8/15/85	3	66	60	49	26	−16
	12/6/85	0.5	51	69	33	37	43
2	5/30/85	0.5	72	83	64	68	41
	10/17/85	2.5	70	65	39	32	8
	12/6/85	1.5	70	81	50	47	20
3	5/2/85	0	68	101	55	63	56
	7/25/85	1	79	77	51	53	−16
	11/22/85	3	78	68	54	40	−26
4	5/2/85	0	66	101	50	50	ND
	10/12/85	1	67	70	54	57	11
	10/17/85	1	71	67	52	50	24
	11/22/85	1	78	84	64	67	ND
5	5/14/85	0	50	80	39	33	ND
	10/17/85	1	76	78	53	52	ND
	11/1/85	1	84	79	37	27	ND
	12/13/85	1	79	78	55	50	24

in the disease activity. Increased expression of the 2H4 molecule was observed on December 6 with decreased disease activity, although the patient was then receiving higher corticosteroid doses than on August 15. These results strongly suggest that the impaired 2H4 expression, as well as the defect in suppression by AMLR-activated T4 cells is not solely due to the effect of steroid treatment.

The precise mechanism of the defective expression of the 2H4 antigen is not clear. It was possible that the defect in 2H4 antigen expression in active SLE could be attributable to altered kinetics of 2H4 antigen expression. Although in normal controls, the density of the 2H4 antigen gradually increased and peaked on day 7, the density of 2H4 in SLE patients progressively decreased after the initiation of the AMLR (Fig. 4). Thus this possibility appears unlikely. Although T4$^+$2H4$^+$ cells generally responded maximally to autologous non-T cells in normals, T4$^+$2H4$^-$ but not T4$^+$2H4$^+$ cells may respond well to autologous non-T cells in patients with active SLE. If so, T4$^+$2H4$^-$ cells may expand more in culture than the T4$^+$2H4$^+$ cells, resulting in a decreased average 2H4 density and percent 2H4$^+$ cells.

Our previous studies showed that the T4$^+$2H4$^-$ helper inducer subset of cells exhibits enhanced Ig synthesis after activation in the AMLR. It is conceivable that in active stages of SLE, T4$^+$2H4$^-$ cells may be preferentially activated in response to self–class II molecules in vivo, and these activated T4$^+$2H4$^-$ cells give an increased helper signal to B cells, thus enhancing production of a variety of autoantibodies. Alternatively, T4$^+$2H4$^+$ cells from patients with active SLE may have already been activated in vivo; after stimulation with autologous non-T cells, the 2H4 antigen expression of T4 cells might be decreasing just as the 2H4 density of the day 7 AMLR T4 cells from normal controls decreased gradually after the maximum expression of the 2H4 antigen on day 7. In this regard, the 2H4 antigen expression on the day 7 AMLR-activated T4 cells from normal controls decreased more rapidly after restimulation of these cells with autologous non-T cells than did 2H4 on day 7 AMLR-activated T4 cells cultured without restimulation (Table III).

Antilymphocyte antibodies in SLE can mediate cell killing in the absence of complement (24, 25) or modulate the expression of surface receptors at physiologic temperatures (26), with consequent alteration in lymphocyte function. Especially, warm-reactive SLE antilymphocyte antibodies of the IgG class have been shown to interfere with normal T cell function (24, 27). Therefore, such antibodies may be important in defective T cell function. In the present study, antilymphocyte antibodies or other serum factors may have interacted with certain T cell surface antigens, resulting in defective expression of the 2H4 molecule after AMLR activation and causing a defect in suppressor function.

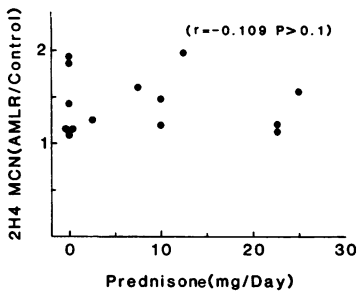

Figure 5. Relationship between the dose of prednisone and the ratio of MCN of 2H4 on AMLR-activated T4 cells to that on control T4 cells from patients with inactive SLE. The dose of prednisone in patients was not correlated with the ratio of MCN of 2H4 on AMLR-activated T4 cells to that on control T4 cells from patients with inactive SLE ($\gamma = -1.09$; $p > 0.1$).

In summary, the present study strongly supports the notion that defective expression of the 2H4 molecule may be an important mechanism for the failure of active SLE patients with normal percentages of $T4^+2H4^+$ cells to generate suppression. The present results lend further strong support to the importance of expression of the 2H4 molecule itself after cell activation in the generation of suppressor activity.

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