Impaired T Cell Capping and Receptor Regeneration in Active Systemic Lupus Erythematosus

EVIDENCE FOR A DISORDER INTRINSIC TO THE T LYMPHOCYTE

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ABSTRACT It is currently unclear whether the T cell dysfunctions observed during active systemic lupus erythematosus (SLE) reflect a disorder intrinsic to the T cell or defects that result from interaction with anti-T cell autoantibody. To determine whether a disorder intrinsic to the T cell exists in SLE, the T cell capping mechanism was selected as a model of cellular function. The normal T cell capping mechanism is a rapid, energy-dependent and coordinated sequence of membrane events that consists of microaggregation, capping, endocytosis, and regeneration of the surface molecule. The monoclonal antibodies OKT3, OKT4, and OKT8, directed against the T cell-specific membrane glycoproteins T-3, T-4, T-8, served as specific probes of the glycoproteins' mobility within the membrane and membrane glycoprotein regeneration. When compared with >91% T cell capping in normal and control subjects with active Sjögren's syndrome, active rheumatoid arthritis and active tuberculosis, only 49–60% of T cells from active SLE patients completed the capping sequence (SLE vs. healthy controls; T-3, $P < 0.002$; T-4, $P < 0.004$; T-8, $P < 0.002$). Colchicine (10^{-5} M), which inhibits microtuble polymerization and augments the rate of normal T cell capping, failed to restore the abnormal capping. However, as judged by the elapsed time intervals to half-maximal capping, the capping kinetics of the T cells able to initiate capping were not significantly different from controls. Fluorescence microscopy demonstrated an abnormal staining pattern characterized by microaggregation of ligand-glycoprotein complexes on resting T cells, coarse aggregation of ligand-glycoprotein complexes over the surfaces of cells that failed to cap, and cleaved or disrupted caps. After clearance of determinants by capping, >94% of T cells from healthy controls regenerated T-3, -4, and -8 within 24 h. In contrast, only 20–40% of capped T cells from active SLE patients reexpressed new determinants. With improving disease activity, the proportion of cells capping and regenerating T-3, -4, and -8 increased, but remained significantly below control levels. In conclusion, this study has identified a disorder of T cell surface glycoprotein mobility and regeneration affecting the majority (60–80%) of both the $T\text{-}3^+$,$T\text{-}4^+$ (inducer/helper), and $T\text{-}3^+$,$T\text{-}8^+$ (suppressor) subsets during active SLE. Although the impaired capping and reexpression improve with disease remission, a residual defect persists. The data support the concept of a disorder intrinsic to the T cell in SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE)[1] is an autoimmune disorder characterized by protean clinical signs and abnormal humoral and cellular immune responses (1). Although the disordered immune responses reflect, in part, impaired control of B and T lymphocyte functions by regulatory T cells, the nature of the regulatory T cell dysfunction remains unclear.

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[1] *Abbreviations used in this paper:* FITC, fluorescein isothicyanate; OKT3, OKT4, OKT8, monoclonal antibodies directed against T-3, T-4, and T-8 receptors; PBMC, peripheral blood mononuclear cells; SLE, systemic lupus erythematosus.

Several T lymphocyte dysfunctions have been identified during active SLE. These include (*a*) reduced T cell proliferation in response to mitogens (2) and antigens (3), (*b*) depressed cell-mediated lympholysis (4), (*c*) deficient autologous mixed lymphocyte responses (5–7), (*d*) impaired elaboration of interleukin 2 and soluble immune response suppressor (8, 9), and (*e*) suppressor T cell dysfunctions (10–15). The coexistence of diverse T cell dysfunctions as well as varying T cell immune competence with disease activity (9, 15) suggested that a disorder intrinsic to the T cell might result in aberrant immunoregulation in SLE.

To investigate the existence of a disorder primary to the T cell in SLE, the capping mechanism was selected as a model of membrane function. The capping process, initiated immediately upon the binding of monoclonal antibody and anti-antibody by T cell-specific surface molecules (T-3, T-4, T-8), consists of a sequence of microaggregation, capping, and endocytosis of ligand-glycoprotein complexes occurring over 30 min at 37°C. After clearance of the surface molecules by capping and endocytosis, new molecules reappear on the cell surface within a period of 24 h (16). Both the capping sequence and the reexpression of new molecules are regulated by the cytoskeleton. Colchicine, an agent that binds irreversibly to tubulin subunits and inhibits microtuble polymerization, augments both the rate of T cell capping and the reexpression of new surface molecules. In contrast, cytochalasin B, an agent that binds to actin filaments and blocks actin polymerization, inhibits both T cell capping and regeneration of surface molecules (16).

Using T cell capping as a sensitive probe of cytoskeleton-membrane interactions, we examined T cell function in SLE at the single-cell level. The kinetics of capping and the capacity to regenerate new surface molecules in SLE were compared with that observed in healthy subjects and in control subjects with other rheumatic diseases (e.g., primary Sjögren's syndrome and rheumatoid arthritis) and an infectious disease (e.g., tuberculosis). The results support the concept of a disorder intrinsic to the T cell in SLE.

METHODS

Patient and control populations. 20 women and one man with the established diagnoses of SLE were studied. The diagnosis of SLE was based upon the following criteria of the American Rheumatism Association for the classification of SLE: (*a*) arthritis, malar rash, photosensitivity, nasal/oral ulcers, pleuritis or pericarditis, seizures and psychosis, proteinuria (>500 mg/d) or cylindruria, hemolytic anemia or leukopenia or thrombocytopenia, false-positive venereal disease research laboratory test (17); (*b*) antinuclear antibody titer ≧ 1:160; and (*c*) anti-native (n)DNA > 15 U/ml by Farr assay.

For the purposes of this study, SLE patients were allocated to one of three groups containing six to nine persons each, on the basis of the state of disease activity when studied: active, mildly active, and inactive. Active disease was defined as the presence of ≧4 criteria, an antinuclear antibody titer > 1:160 and anti-nDNA > 35 U/ml. Mildly active disease was designated as the presence of <4 criteria, an antinuclear antibody titer > 1:40 but not necessarily the presence of circulating anti-nDNA. Inactive disease was defined as clinical remission as judged by the absence of clinical disease for an interval of at least 3 mo. All subjects with either mildly active or inactive disease had previously fulfilled the clinical and serologic criteria for active SLE.

Active SLE patients were studied while hospitalized during the peak of clinical activity, but before the initiation of any therapy. The remainder of subjects were studied either as outpatients or inpatients of the Clinical Research Center of University Hospitals of Cleveland. Although several of these patients were being managed with aspirin, nonsteroidal antiinflammatory agents, or hydroxychloroquine, these agents were discontinued >72 h before study. Blood samples from two patients treated with ≦10 mg/d prednisone were obtained 24 h after administration of the drug (18, 19). No study subject had received immunosuppressive agents within the previous 30 d. All signed an informed consent for human investigation and agreed to venipuncture.

Four control groups were selected. As controls for autoimmune disease, six sex-matched individuals (five females, one male) with active primary Sjögren's syndrome were studied. The established criteria for this disorder include (*a*) keratoconjunctivitis sicca and (*b*) abnormal labial gland biopsy (20). As a second group of controls for autoimmune disease, six sex-matched persons (five females, one male) with classical or definite rheumatoid arthritis (21) were selected. All patients were functional class IV and exhibited active polyarticular synovitis when studied. The third control group consisted of six males with an infectious disease, tuberculosis. The diagnosis was confirmed in each case by culture of *Mycobacteria tuberculosis*. When studied, three patients had not yet received therapy and the remainder had been treated with isoniazid, ethambutol, or rifampin for ≦2 wk. 12 age- and sex-matched healthy persons taking no medications served as normal control subjects. The clinical characteristics of the SLE patients, and the Sjögren's, rheumatoid arthritis and tuberculosis control subjects are summarized in Table I.

Reagents. The monoclonal antibodies OKT3, OKT4, and OKT8 were obtained in an azide-free, lyophilized form (Ortho Pharmaceuticals, Raritan, NJ). $F(ab')_2$ goat anti-mouse IgG (anti-antibody) and fluorescein isothiocyanate (FITC)-anti-antibody were purchased from Cappel Laboratories, Cochranville, PA. Colchicine was obtained from Sigma Chemical Co., St. Louis, MO.

Cell preparation. Peripheral blood mononuclear cells (PBMC) were separated from heparinized venous blood by Ficoll/Metrizoate (Accurate Chemical & Scientific Co., Westbury, NY) density gradient centrifugation. To dissociate cytophilic antibody from the T cell surface and to eliminate the majority of monocytes, PBMC were incubated on plastic surfaces for 60 min at 37°C. After recovery of the nonadherent PBMC, an enriched T lymphocyte preparation was obtained by sheep erythrocyte rosetting as previously described (16). The resultant cell preparation was composed of >98% viable T lymphocytes, as determined by staining with OKT3/FITC-anti-antibody and ethidium bromide-acridine orange supravital stain. With FITC-$F(ab')_2$ goat anti-human Ig (Cappel Laboratories), <1% of control and SLE

TABLE I
Characteristics of the SLE and Control Populations

Patient	Race/Sex/Age*	Disease duration‡	Disease manifestations when studied§	Activity/treatment‖
Systemic lupus erythematosus				
1	B/F/39	2	A, An, L, Ray	MA/none
2	C/F/19	1	A, An, L, OU, Poly, R, Ray, T	A/none
3	C/F/23	10	none	IA/HC, NSAID
4	B/F/46	8	A, R	MA/NSAID
5	C/F/27	1	A, An, Gn, L, Poly, R, Vasc	A/none
6	B/F/25	3	none	IA/none
7	C/F/32	4	A, Alo, An, H, L, Poly, R, T	A/none
8	C/F/27	9	A, Poly, Ray	MA/HC, NSAID, Pred
9	C/F/19	1	None	IA/ASA
10	C/F/64	0.5	Alo, An, BFP, L, M, PS, R, Ray	A/none
11	B/F/35	2	A, Alo, Poly, L	MA/none
12	B/M/39	1.5	GN, Poly, R	MA/HC, Pred
13	B/F/36	3.5	Alo, An, GN, L, M, N, Psy, R	A/none
14	C/F/20	1.5	None	IA/none
15	C/F/30	5	R	MA/none
16	C/F/25	1.5	None	IA/none
17	C/F/31	5	A, An, GN, L, OU, PS, R, Ray	A/none
18	C/F/58	26	BFP	IA/none
19	B/F/42	1	A, Alo, An, L, R	A/none
20	C/F/36	0.5	A, An, Gn, Poly, P, Ray	A/none
21	C/F/29	17	A, An, Gn, OU, R, T, Vasc	A/none
Primary Sjögren's syndrome				
22	C/F/61	2	S	A/HC
23	C/F/55	3	S	A/HC
24	C/F/21	2	S	A/none
25	C/M/49	14	S	A/HC
26	B/F/34	5	S	A/none
27	C/F/42	9	S	A/none
Rheumatoid arthritis				
28	C/F/36	4	Syn	A/NSAID
29	C/M/67	10	No, Syn	A/NSAID, PCM
30	C/F/41	6	Syn	A/NSAID, G
31	C/F/60	32	No, Syn	A/NSAID
32	C/F/57	11	Syn	A/none
33	C/F/43	6	Syn	A/none
Tuberculosis				
34	B/M/42	<2 wks	Pulm	A/I, R
35	B/M/66	<2 wks	Pulm	A/I, R
36	C/M/50	<2 wks	Pulm	A/none
37	C/M/52	1 wk	Pulm	A/none
38	B/M/26	<1 wk	Pulm	A/none
39	C/M/39	1 wk	Pulm	A/E, I

* Mean ages (range): active SLE, 35 yr (19–64 yr); mildly active SLE, 36 yr (27–46 yr); inactive SLE, 28 yr (19–58 yr); Sjögren, 44 yr (21–61 yr); rheumatoid arthritis, 51 yr (36–67 yr); tuberculosis, 46 yr (26–66 yr).

‡ Mean disease duration (range): active SLE, 2.5 yr (0.5–5 yr); mildly active SLE, 4.6 yr (1.5–9 yr); inactive SLE, 7.2 yr (1–26 yr); Sjögren, 5.8 yr (2–14 yr); rheumatoid arthritis, 12 yr (4–32 yr); tuberculosis, (1–2 wk).

§ A, arthritis; Alo, alopecia, An, anemia; BFP, biological false positive venereal disease research laboratory test; GN, glomerulonephritis; H, headaches; L, leukopenia; M, myositis; N, neuropathy; No, nodules; OU, oral/nasal ulcers; Poly, polyserositis; Pulm, pulmonary disease; PS, photosensitivity; Psy, psychosis; R, rash; Ray, Raynaud's; S, sicca complex; Syn, synovitis; T, thrombocytopenia, Vasc, vasculitis.

‖ A, active diseases, MA, mildly active SLE; IA, inactive SLE. ASA, aspirin; E, ethambutol; G, gold salts; HC, hydroxychloroquine; I, Isoniazid; NSAID, nonsteroidal antiinflammatory drugs; PCM, penicillamine; Pred, Prednisone, R, Rifampin.

T cells were fluorescent, indicating the absence of cytophilic antibody or cell-bound immune complexes.

Capping of T cell-specific determinants. The capping technique was carried out as previously described (16). In brief, 5×10^5 enriched T cells were centrifuged through HBSS (Hanks' buffered salt solution; Gibco Laboratories, Grand Island, NY) containing 5% bovine serum albumin (BSA, Fraction V, Miles Laboratories Inc., Elkhart, IN) in 6×50-mm glass test tubes at 400 *g* for 5 min at 4°C. Cell pellets were then resuspended in monoclonal antibody (OKT3, OKT4, or OKT8), 625 ng/ml, 50 μl, and incubated at 4°C for 30 min with interval gentle resuspension. After washing with HBSS supplemented with 0.3% BSA, the pellets were resuspended in FITC-anti-antibody 1:20, 50 μl, at 4°C for 30 min in the dark. The cells were washed, resuspended, allowed to settle at 4°C for 15 min, and then transferred to a 37°C water bath for performance of the kinetic studies. Capping was stopped by prompt fixation of cells in an equal volume of 2% paraformaldehyde. The proportion of cells capped at 0-, 2-, 5-, 10-, 15-, 20-, and 30-min intervals was enumerated to estimate the rate of capping of the T cell-specific determinants. The effect of colchicine upon the capping mechanism was investigated by the protocol previously described (16).

A cell was designated capped if there was polar fluorescence covering less than half of the cell surface, and was said to have endocytosed the ligand-glycoprotein complexes if three or fewer fluorescent vesicles were visualized within the cytoplasm. To verify that caps were localized to the external surface of the membrane and that fluorescent vesicles were intracellular, 10 mM isotonic cupric sulfate replaced the medium bathing the cells. Fluorescence emitted by a source on the external surface of the cell is quenched by the cupric ion, whereas that from an intracellular source is not quenched (Dr. Dean Hafeman, personal communication; reference 22). For purposes of enumeration, capped cells included those exhibiting either capping or endocytosis. However, to limit the subjectivity of interpretation, capping experiments were carried out with both a control and a SLE study subject in a blinded fashion. A minimum of 200–300 cells was enumerated for each data point.

Estimation of the time intervals to half-maximal capping. To compare the rates of capping of T-3, -4, and -8 of SLE and control T cells, the time required to achieve half-maximal capping was estimated. Since previous experiments had demonstrated that the kinetic curves generated by normal T cell capping were asymptotic over a 30-min interval, the model of exponential decay kinetics was applied (16). The following equation expresses the relationship between time and the percent capped cells: $\log (N - Y) = -\log N\ kt$, where N represents the limiting percentage of capped T cells at 30 min, Y is the percentage of capped cells at a given time, k is the rate constant, and t is time. The time intervals to half-maximal capping were estimated from the linear curves generated by plotting the $\log (N - Y)$ vs. time in minutes.

Rate of reexpression of T cell determinants. The capacity of T cells to regenerate surface T cell-specific determinants after capping was examined at 2, 14, and 24 h, as previously detailed (16). 5×10^5 T cells were incubated with OKT3, OKT4, or OKT8, followed by the anti-antibody. After resuspension in RPMI 1640 (Gibco Laboratories) supplemented with 10 mM Hepes, 2 mM L-glutamine, 10 μg/ml gentamicin, and 10% pooled, heat-inactivated AB serum (gift of Dr. Louise Keating, Red Cross, Cleveland, OH), the cell preparations were incubated for 60 min at 37°C to promote capping and endocytosis of ligand-glycoprotein complexes. The capped cells were washed and then cultured for intervals of 2, 14, and 24 h at 37°C in 5% CO_2 to permit reexpression of new glycoprotein molecules. Upon harvesting, cell preparations were immediately chilled in media containing sodium azide (10^{-1} M), rinsed twice, restained with the monoclonal antibodies and FITC-anti-antibody, and fixed. Similarly treated control cell preparations were stained with acridine orange-ethidium bromide, and the proportion of viable cells enumerated. After adjusting for cell viability, the proportion of T cells capable of regenerating the specific determinant was calculated by enumerating the percentage of 300 fluoresceinated cells exhibiting a circumferential rim of fluorescence. Cells that had failed to replenish surface determinants did not show rim fluorescence, but rather still showed capping or endocytosis. In contrast, cells that had not capped displayed variable aggregation of ligand-glycoprotein complexes, as described in Results.

Statistics. Statistical significance ($P < 0.05$) was calculated by the Fisher's exact test or by one-way analysis of variance.

RESULTS

Impaired capping of T cells from patients with active SLE. The capacity of T cells from patients with active SLE to cap the T-3, -4, and -8 determinants was markedly impaired. Fig. 1, which depicts the proportion of T cells capped at specific time intervals over 30 min, compares T cell capping in patients with active, mildly active, and inactive SLE with control subjects. Only 49–60% of T cells from active SLE patients completed the capping sequence within the 30-min interval. When compared with >95% T cell capping in healthy controls, the percentage of capped cells was significantly reduced (T-3, $P < 0.002$; T-4, $P < 0.004$; T-8, $P < 0.002$). Moreover, permitting the SLE T cells to cap over a 60-min rather than a 30-min period did not significantly enhance capping (Table II).

To determine whether the kinetics of T cell capping during active disease lagged behind controls, the elapsed time intervals to half-maximal capping were calculated and contrasted to controls. The data shown in Table III demonstrate that, in those cells able to initiate the capping sequence, the times to achieve half-maximal capping were not significantly different from controls. This was true of both the $T\text{-}3^+$,$T\text{-}4^+$ (inducer/helper) and $T\text{-}3^+$,$T\text{-}8^+$ (suppressor) subsets.

Altered surface morphology of T cells by immunofluorescence during capping. The surface staining of T cells from patients with active disease was altered, as judged by immunofluorescence. In contrast to normal, resting T cells, which possessed a smooth, thin rim of fluorescence, resting T cells from patients with active SLE often exhibited marked microaggregation of the ligand-glycoprotein complexes (Fig. 2 *A*). This pattern of surface staining was observed on 50–80% of cells compared with 0–9% of normal control cells stained with OKT3, OKT4, or OKT8. The microag-

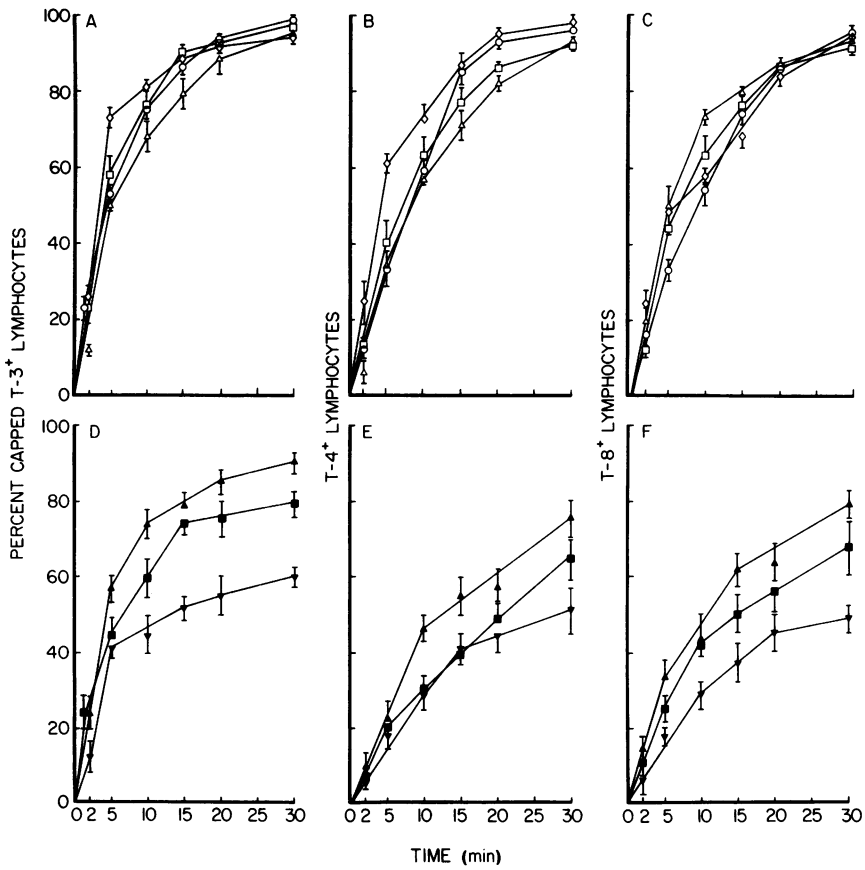

FIGURE 1 The kinetics of capping of T-3 (*A*, *D*), T-4 (*B*, *E*), and T-8 (*C*, *F*) at 37°C over time intervals to 30 min. Comparison is made between healthy controls (○), Sjögren's controls (◇), rheumatoid arthritis controls (△) and tuberculosis controls (□) (*A–C*), and patients with active SLE (▼), mildly active SLE (■), inactive SLE (▲) (*D–F*).

gregation resembled the patching of ligand-glycoprotein complexes sometimes observed early (1–2 min) in the normal capping sequence (16, 23), but was dissimilar in that it occurred during the resting state and often consisted of larger aggregates.

Cap morphology was frequently abnormal as well.

TABLE II
Comparison of Mean Percentage of Capped T Cells from Patients with Active Disease at 30 and 60 min

T cell determinant	Percentage capped±SEM	
	30 min	60 min
T-3	60±5.0	68±4.0
T-4	51±7.5	61±5.0
T-8	49±4.4	55±6.1

n = 6 patients.

Instead of the formation of a compact cap with a smooth surface, >85% of caps were cleaved or fragmented and had irregular surfaces (Fig. 3). The defective cap architecture was observed on >80–85% of cells of both T cell subsets during active disease. In contrast, the frequency of such cap morphology was 8% (range, 0–12%) in all control groups except tuberculosis, in which it was somewhat higher (mean, 11%; range, 3–16%).

T cells that had failed to initiate capping within 15–30 min frequently exhibited large, irregular, and globular aggregates of ligand-glycoprotein complexes (Fig. 2 *D*). For the sake of description, these have been designated macroaggregates. By means of cupric sulfate, the macroaggregates were localized to the external surface of the cells. When compared with only 2% (range, 0–5%) of normal T cells that exhibit macroaggregates, >80% of uncapped T cells of both subsets possessed such macroaggregates. To determine whether

TABLE III

*Elapsed Time Intervals to Half-maximal Capping of T-3, T-4, and T-8 in Patients with SLE and Controls**

Study group	Surface determinant	Time interval to half-maximal capping
		min
Active SLE‡	T-3	4.0±1.4
	T-4	8.4±1.7
	T-8	7.6±1.1
Mildly active SLE‡	T-3	4.2±0.5
	T-4	8.2±0.8
	T-8	7.2±1.0
Inactive SLE‡	T-3	3.5±1.8
	T-4	8.9±1.3
	T-8	6.9±1.6
Sjögren controls	T-3	2.2±0.9
	T-4	6.6±1.0
	T-8	6.1±0.9
Rheumatoid arthritis	T-3	5.2±0.2
	T-4	6.7±0.7
	T-8	5.1±0.3
Tuberculosis	T-3	3.5±0.8
	T-4	5.2±0.4
	T-8	4.1±0.9
Healthy controls	T-3	4.6±0.9
	T-4	6.1±1.1
	T-8	7.1±0.6

* To compare the rates of capping of T-3, -4, and -8 for each of the study groups, the model of exponential decay kinetics was used. The elapsed time intervals to half-maximal capping were estimated from the linear curves generated by plotting the log $(N - Y)$ vs. time.
‡ Comparison of time intervals of patients groups to controls showed no significant difference.

such cells would eventually cap and endocytose the aggregated complexes, cells were incubated at 37°C for intervals to 4 h. Cells that had not completed the capping process within 30–45 min in general could not clear the macroaggregates from their surface.

Partial reversibility of impaired capping during mildly active and inactive SLE. Although the proportion of T cells that capped within the 30-min interval progressively increased as disease activity waned (Fig. 1), the capping defect appeared to be only partially reversible. T cells from patients with mildly active and inactive SLE capped 65–75% and 75–90%, respectively. When compared with normal controls, these percentages of capped T cells were still often significantly reduced (mildly active SLE vs. normals: T-3, $P < 0.004$; T-4, $P < 0.004$; T-8, $P < 0.005$; inactive SLE vs. normals: T-3, NS: T-4, $P < 0.008$; T-8, $P < 0.02$). However, a smaller proportion of cells from patients with inactive disease exhibited microaggregation (mean, 23%; range, 9–28%), defective caps (mean, 29%; range, 3–34%) or macroaggregation (mean, 12%; range, 6–22%).

Impaired regulation of capping by the cytoskeleton. To establish whether the impaired capping mechanism could be reversed by an agent that augments normal T cell capping by modifying microtubular organization, T cells from controls or patients with SLE (active SLE, $n = 5$; mildly active SLE, $n = 6$; inactive SLE, $n = 6$) were briefly pretreated with colchicine. After washing away the agent, the cells were bound with monoclonal antibody and FITC-anti-antibody and kinetic studies were performed over a 10-min interval. When compared with cells preincubated with media, a significantly increased percentage of both normal and Sjögren T cells pretreated with colchicine capped (T-3: Sjögren, $P < 0.01$; normals, $P < 0.04$) (Fig. 4 *A*). Similar results were obtained with T-4 and T-8 (data not shown). These findings point out that there is no apparent cytoskeletal dysfunction of T cells from Sjögren patients and that such T cells behave like normal cells. By comparison, colchicine-pretreated T cells from active SLE patients exhibited a decrement in the proportion of capped T 3 cells (Fig. 4 *B*) (comparison of colchicine- vs. media-preincubated cells, $P < 0.003$). Similar findings were also observed with T-4- and T-8-capped cells (data not shown). However, T cells from patients with inactive disease partially regained their capacity to respond to colchicine. Although the proportion of capped T cells from mildly active SLE patients neither increased nor decreased in response to colchicine (Fig. 4 *C*), the agent appeared to augment T cell capping during inactive disease (Fig. 4 *D*), but not to a significant degree (comparison of colchicine- vs. media-preincubated cells, $P < 0.3$). Parallel observations were made with T-4- and T-8-capped cells (data not shown). These experiments demonstrate that regulation of the capping mechanism by cytoskeletal proteins was impaired during active SLE and could not be restored by an agent that augments the rate of normal T cell capping. During intervals of lesser disease activity, the capacity of T cells to respond to colchicine by enhanced capping seemed to improve progressively; however, the response to colchicine did not approach control values.

Impaired reexpression of the T-3, T-4, and T-8 determinants during active SLE. Regeneration of T-3, -4, and -8 on a population of normal capped T cells gradually occurs over 24 h (16). To establish whether T cells from patients with active SLE were capable of effective regeneration of these determinants, capped cells were cultured for various intervals

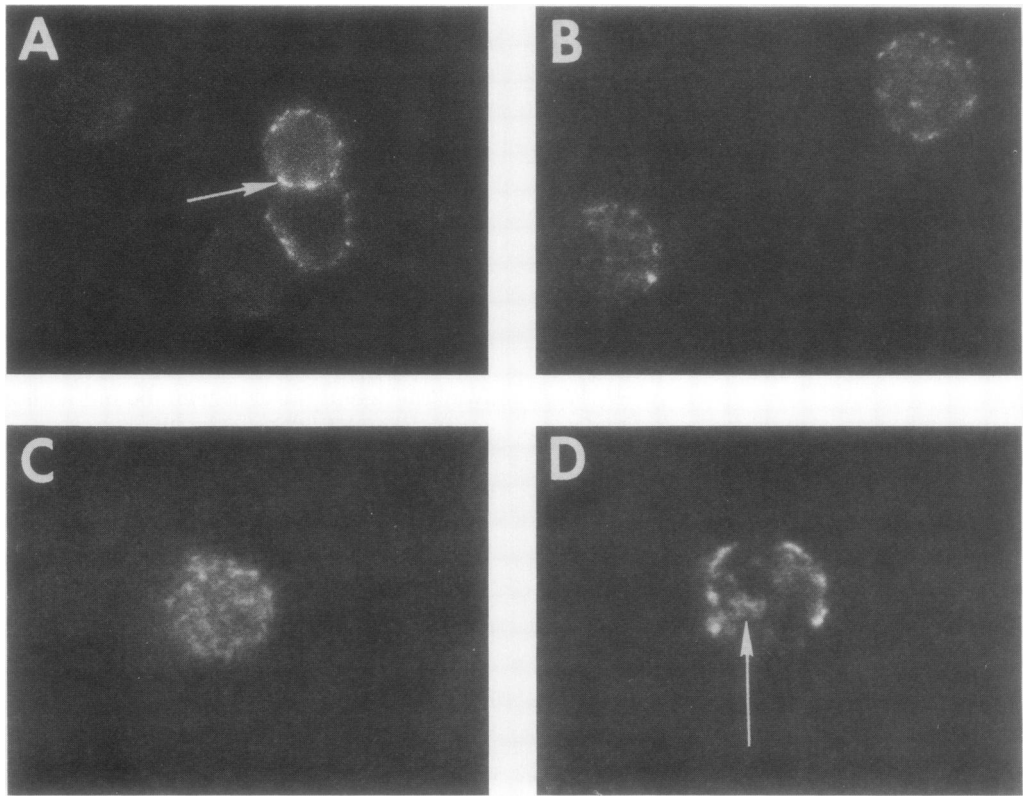

FIGURE 2 Surface morphology of T cells by immunofluorescence during active SLE. (*A*) Resting T cells exhibited microaggregation of T-3, -4, or -8 surface molecules (arrow). (*B*) 2-min incubation. The left cell has initiated cap formation, but has retained its microaggregates and developed early clumping of surface molecules. The right cell has not initiated capping, still possesses microaggregates, and has developed early clumping of molecules. (*C*) 10-min incubation. This cell has not initiated capping and shows coarse aggregates of surface molecules. (*D*) 30-min incubation. This cell has failed to cap. Instead, the cell exhibited macroaggregation of surface molecules (arrow), which persisted on the cell surface and were not endocytosed.

over 24 h, restained, and the proportions of viable cells bearing rim fluorescence were enumerated. The results showed that the capacity of T-cells to regenerate T-3, -4, and -8 was significantly impaired when compared with healthy controls (T-3: 2 h, $P < 0.04$; 14 h, $P < 0.00004$; 24 h, $P < 0.00007$; T-4: 2 h, $P < 0.001$; 14 h, $P < 0.000003$; 24 h, $P < 0.000001$; T-8: 2 h, $P < 0.08$; 14 h, $P < 0.004$; 24 h, $P < 0.00004$) (Fig. 5). As shown in Fig. 5, the majority of T cells failed to reexpress new surface molecules, especially at 14 and 24 h. This was not the result of either cell loss or cell death, for cell recovery at 24 h was >88% and cell viability was >90%, as judged by ethidium bromide-acridine orange staining.

By comparison, capped T cells from patients with mildly active or inactive SLE exhibited a higher proportion of cells capable of regeneration of T-3, -4, and -8. However, when compared with normal controls, T cells from persons with mildly active disease still did not regenerate the molecules effectively (T-3: 2 h, $P < 0.002$; 14 h, $P < 0.001$; 24 h, $P < 0.0001$; T-4: 2 h, $P < 0.02$; 14 h, $P < 0.002$; 24 h, $P < 0.0002$; T-8: 2 h, $P < 0.01$; 14 h, $P < 0.006$; 24 h, $P < 0.0001$). Although inactive disease was associated with still more capacity to reexpress new determinants, there remained a significant lag at 24 h (T-3: $P < 0.01$; T-4, $P < 0.002$; T-8, $P < 0.001$). Although gradual recovery of regeneration of T-3, -4 and -8 appears to be associated with resolution of disease activity, a residual defect in the reexpression of new determinants persists.

Identification of functionally normal and abnormal T cell populations. The dissociation between the cells exhibiting normal intervals to half-maximal capping and those unable to initiate capping indicated that there were at least two populations of T cells. The former

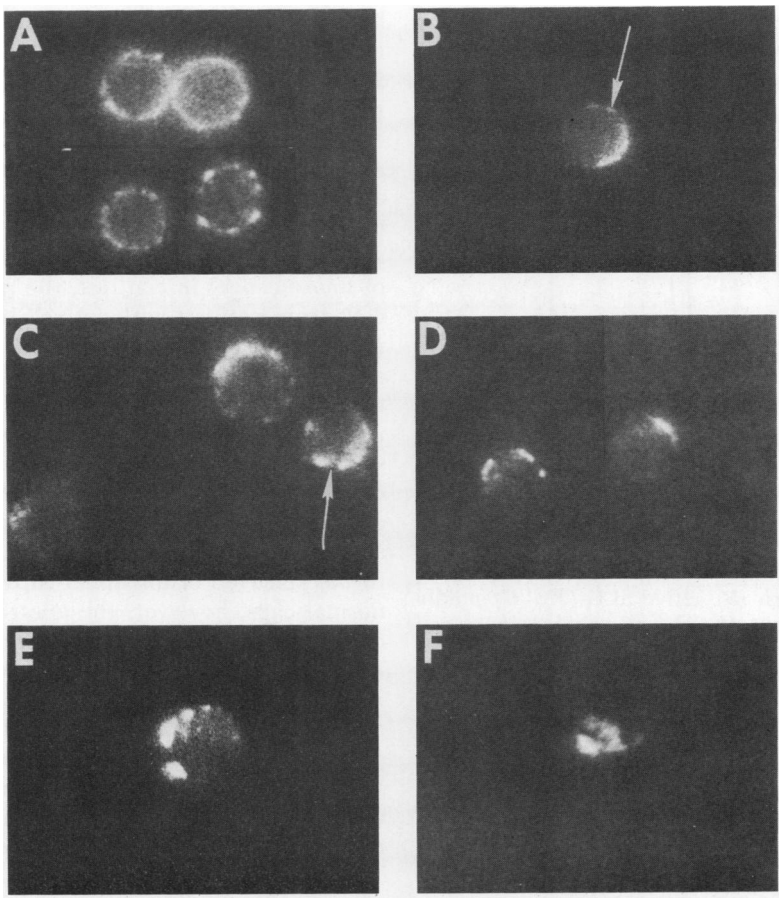

FIGURE 3 Surface morphology of resting and capped T cells by immunofluorescence during active SLE. (*A*) This panel contrasts the rim fluorescence staining pattern with that of microaggregation of surface molecules. This pattern of surface staining was observed on 50–80% of cells compared with 0–9% of control cells. Note the variable size of the microaggregates among cells. Patients with active SLE often possessed T cells with both normal and anomalous staining patterns. (*B*) 2-min incubation; early cleaved cap (arrow). (*C*) 5-min incubation; cleaved or disrupted cap (arrow). Note the clumped appearance and irregular surface of the cap. The center cell also exhibited a clumped appearance and irregular surface. Note the retained fluorescent microaggregates that have failed to be capped. (*D*) 10-min incubation. Two capped cells are shown. The left cell typifies the disrupted cap. The right cell possesses a formed, compact cap. Both normal and abnormal capped T cells were often observed. (*E*) 15-min incubation; disrupted cap. (*F*) 30-min incubation: T cell with disrupted cap that has not yet undergone endocytosis.

population is composed of ~60% of T-3$^+$ cells and 50% of T-4$^+$ and T-8$^+$ cells, respectively (Fig. 1). However, of these only ~40% of T-3$^+$, 20% of T-4$^+$, and 34% of T-8$^+$ cells reexpressed their respective determinants (Fig. 5). This finding indicated that although approximately one-half of T cells could be induced to cap, variable proportions could not regenerate determinants and were therefore not functionally normal cells. The second population of T cells, composed of ~40% of T-3$^+$, and 50% of T-4$^+$ and T-8$^+$ cells, respectively (Fig. 1), failed to initiate the capping sequence upon binding the ligand. Thus, the cellular dysfunctions involved 60–80% of T cells and was not restricted to a specific T cell subject.

DISCUSSION

Impaired capping and reexpression of T-3, T-4, and T-8 during active SLE implicate a T cell disorder affecting membrane function and regeneration of surface molecules. Although the dysfunction affects both the T-3$^+$,T-4$^+$ (inducer/helper) and T-3$^+$,T-8$^+$ (sup-

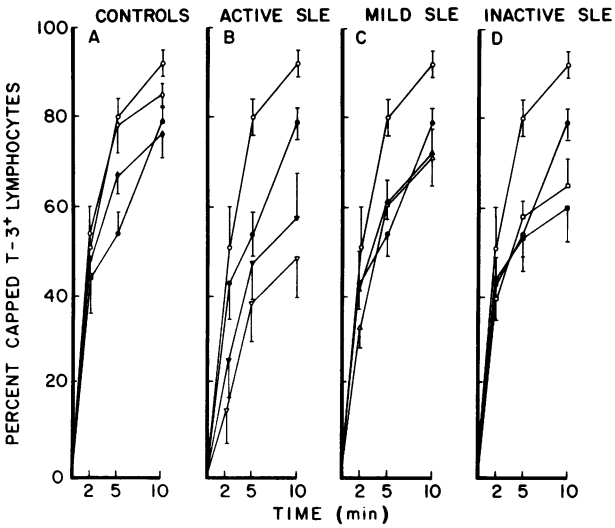

FIGURE 4 The effect of colchicine upon the kinetics of capping. Enriched T cells from (*A*) normal (○) or Sjögren (◇) controls; or patients with (*B*) active SLE (▼); (*C*) mildly active SLE (▲); and (*D*) inactive SLE (■) were preincubated with media (closed symbols) or colchicine (10^{-5} M) (open symbols) for 30 min at 37°C. The cells were then rinsed free of the agent, and treated with OKT3 and FITC-anti-antibody, as detailed in Methods. Kinetics were carried out at 37°C for intervals to 10 min. In panels *B–D*, comparison is made between the patient group and normal controls. (*A*) Colchicine pretreatment of T cells from both control groups significantly augmented the proportion of capped cells. (*B*) In contrast, similar treatment of T cells from persons with active SLE produced a paradoxical decrement in the percentage of capped cells. (*C*) During mild SLE, colchicine neither augmented nor diminished the proportion of capped cells. (*D*) During inactive disease, the capacity of colchicine to augment capping was partially restored.

pressor) subsets, the disorder does not appear to be generalized. Analysis of the elapsed time intervals to half-maximal capping indicated that the minority of T cells that capped did so at a rate similar to controls. Affected T cells failed to initiate the capping mechanism; even after prolonged intervals in culture, the cells could not be induced to cap (Fig. 1). Yet, of the T cells that did cap (Fig. 1), a variable percentage did not reexpress new determinants (Fig. 5). Thus, this model of T-cell function indicates that the majority (60–80%) of T cells are dysfunctional.

This failure of the capping mechanism during active disease contrasts sharply with the rapid onset and completion of the capping sequence by the control T cells. Of particular interest is the observation that the T cells from patients with Sjögren's syndrome, rheumatoid arthritis, and tuberculosis behaved similarly to healthy T cells, indicating that these disorders, and in particular Sjögren's syndrome (which can be associated with SLE), are not characterized by a defect of the T cell capping mechanism. However, there does appear to be a subtle disorder of reexpression of T-4 in tuberculosis and of T-4 and T-8 in Sjögren's syndrome at 24 h (Fig. 5). The significance of this observation remains uncertain.

The abnormal capping mechanism of T cells during active disease was consistently associated with striking alterations of cell surface morphology by immunofluorescence. Ordinarily, the majority of resting T cells from the controls displayed a thin, circumferential rim of fluorescence. In contrast, the majority of resting T cells from patients with active SLE exhibited marked patching, or microaggregation, of ligand-glycoprotein complexes (Figs. 2 *A* and 3 *A*). The majority of cells that did not initiate capping developed globular macroaggregates of ligand-glycoprotein complexes. As shown by the capacity of cupric sulfate to quench fluorescence, these aggregates were scattered over the cell surface and could not be endocytosed over time (Figs. 2, *B–D*). Moreover, capped T cells frequently formed cleaved or disrupted caps (Figs. 3, *B–F*). Like normal caps, however, the aberrant caps were eventually cleared from the membrane by endocytosis. Although microaggregation has been observed in murine T lymphoma cells (24), the constellation of micro- and macroaggregation and disrupted caps has not been previously described in active SLE. However, the full significance of these atypical immunofluorescent patterns and their relationship to the impaired capping response remains to be established.

The defective capping and impaired reexpression of new determinants appeared to reverse partially as disease activity waned. There was an inverse relationship between the state of the disease activity and the proportion of cells capping and regenerating determinants (Fig. 5). However, a residual defect persisted, for T cells from patients with inactive disease neither capped nor reexpressed T-3, -4, and -8 as effectively as did controls (Figs. 1 and 5). It should be emphasized, however, that these studies were carried out with three groups of SLE patients that differed only in disease activity when studied. It is conceivable that evaluation of a single population of SLE patients during varying states of disease activity might yield different results. Yet, based upon limited follow-up of the patient group with active SLE, a similar relationship between less disease activity and augmented capping and reexpression holds. Moreover, the abnormal cell surface architecture also appears to reverse, for T cells from inactive SLE patients less frequently exhibited microaggregation, disrupted caps, or macroaggregation.

Although the mechanisms giving rise to the aberrant capping mechanism and altered reexpression of T-3, -4, and -8 during active SLE remain uncertain, certain potential ones could be operative. Autoantibody directed against T cell surface determinants could in-

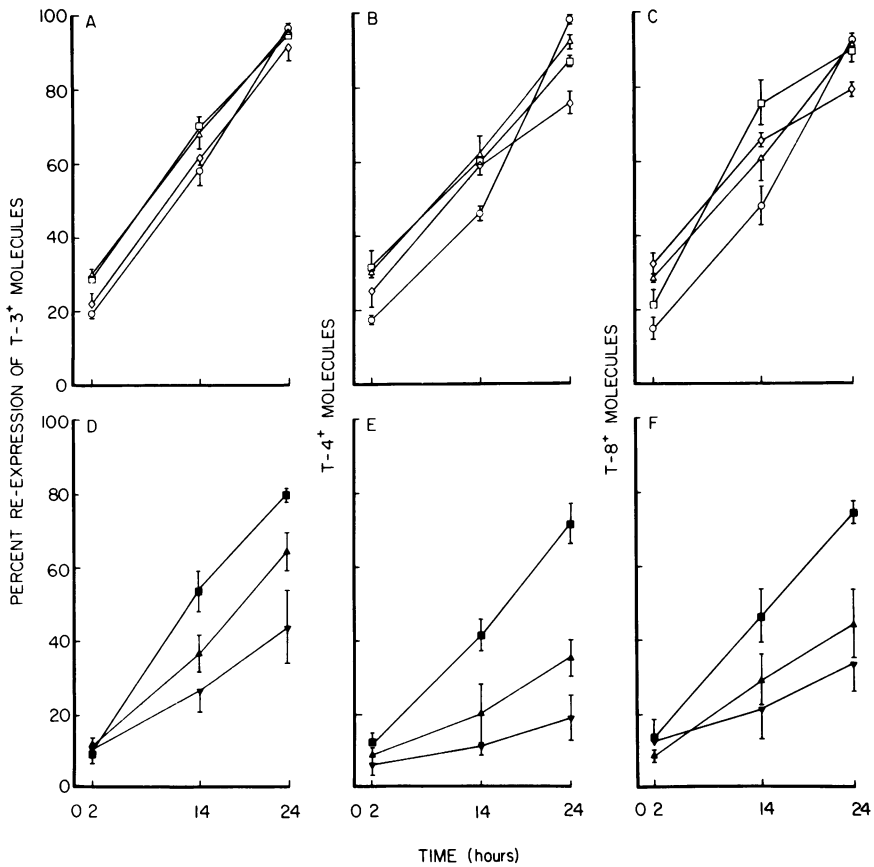

FIGURE 5 Reexpression of T-3 (*A*, *D*), T-4 (*B*, *E*), and T-8 (*C*, *F*) over time. Enriched T cells from healthy controls (○), Sjögren's controls (◇), rheumatoid arthritis controls (△), tuberculosis controls (□) (*A–C*) and patients with active SLE (▼), mildly active SLE (▲) and inactive SLE (■) (*D–F*) were treated with monoclonal antibody and anti-antibody. The cells were incubated for 60 min at 37°C to clear the membrane of the specific glycoprotein molecule, washed, and cultured for intervals of 2, 14, or 24 h in media, as outlined in Methods. Upon harvesting, cell preparations were chilled, rinsed, restained with the appropriate monoclonal antibody and FITC-anti-antibody, and fixed. Cells were tested for viability by staining with acridine orange-ethidium bromide. After adjusting for cell viability, the percentage of 300 fluorescent cells staining with a circumferential rim of fluorescence was enumerated. Cells staining with this immunofluorescent pattern were determined to have regenerated the specific receptor (16).

terfere with cellular function. It is well known that the binding of antibody to surface determinants can alter the immune function of B- and T-cells in both the human and murine systems (25–28). Since the cytoskeleton regulates the mobility of T-3, -4, and -8 (16), anchorage modulation of these molecules by autoantibody could disrupt the capping mechanism. Anchorage modulation is the inhibitory effect of bound concanavalin A on the formation of microaggregates and subsequent caps by other cross-linked membrane receptors due to interactions between such receptors and the cytoskeleton (reviewed in reference 29). To date, only multivalent concanavalin A has been used to examine anchorage modulation in various cell types (30–33). However, it is unlikely that the binding of divalent anti-T cell autoantibody blocked the initiation of the capping sequence by this mechanism since (*a*) neither cytophilic antibody nor cell-bound immune complexes were detected, (*b*) marked microaggregation was observed on the majority of resting T cells, and (*c*) a population of cells could effectively initiate the capping sequence. Moreover, anchorage modulation by autoantibody should not limit the reexpression of new determinants. A second potential mechanism that might alter surface glycoprotein reexpression is autoantibody-mediated suppression. Such inhibition of membrane Ig regeneration has been observed in human peripheral blood B cells, but not splenic or tonsil B cells, capped

with anti-IgM or anti-IgD. It has been proposed that, in contrast to mature splenic or tonsil B cells, anti-Ig-induced clearance of membrane Ig conveyed a negative signal to the relatively immature blood B cell, suppressing regeneration of membrane Ig (34). However, normal circulating T lymphocytes are fully mature cells (35), and preliminary evidence in SLE suggests that only a very small percentage of peripheral T cells bear thymocyte differentiation antigens (Kammer, G.; unpublished data). Thus, antibody-induced clearance should not impair turnover of surface molecules to the extent that has been observed. Yet, mature capped T cells cultured in the presence of the monoclonal antibody used to cap the determinant failed to reexpress that determinant in vitro (16). While the presence of anti-T cell autoantibody could generate a signal suppressing surface glycoprotein reexpression in vivo, the absence of cytophilic antibody or cell bound immune complexes on T cells from SLE patients after incubation should permit effective regeneration in vitro. Thus, although indirect evidence appears to exclude the effect of autoantibody upon the cell membrane as a mechanism for abnormal capping, studies are currently examining this issue.

Since both the T cell capping sequence and regeneration of determinants are regulated by the cytoskeleton (16), abnormal cytoskeletal functions and/or altered membrane glycoprotein-cytoskeleton interactions could effectively interfere with such membrane functions. There is evidence for a link between actin filaments and membrane Ig in murine B cells, and indirect evidence for transmembrane nexuses between other surface receptors and actin- and myosin-containing contractile elements (36, 37). Although there is as yet no direct evidence to support such a mechanism, the observation that colchicine produced an inverse effect upon T cell capping during active, but not inactive disease (Fig. 4), provides preliminary indirect evidence that anomalous cytoskeletal and/or cytoskeleton-receptor interactions may contribute to the disordered T cell receptor regulation.

Lastly, impaired capping and ineffective regeneration of T-3, -4, and -8 could be the product of one or more altered intracellular biochemical pathways. We have demonstrated that brief exposure of normal $T\text{-}3^+$,$T\text{-}4^+$ (inducer/helper) subsets to adenosine triggers a rapid burst of cellular cyclic (c)AMP, the subsequent occupancy of Type I protein kinase receptors by cAMP (38), and a shift in phenotype (RFc_γ^-, $T\text{-}4^+ \rightarrow RFc_\gamma^+$, $T\text{-}8^+$) and development of suppressor activity (39, 40). In contrast, treatment of the $T\text{-}3^+$,$T\text{-}4^+$ subsets from patients with active SLE with adenosine produced a rapid fall in cAMP levels and no immediate changes in cAMP receptor occupancy (38). In turn, there was no subsequent shift of phenotype nor development of suppressor function (15, 38). Thus, the $T\text{-}3^+$,$T\text{-}4^+$ subsets from active SLE patients appear to lack functional adenosine receptor-coupled adenylate cyclase activity. The relationship between the impaired capping mechanism, the capacity to reexpress new surface determinants and altered cAMP-dependent pathways remains uncertain.

In conclusion, this study has identified a disorder of T cell capping and reexpression affecting certain surface molecules of both T cell subsets during SLE. Although the defects partially resolved with disease remission, a residual disorder persisted. The coexistence of this abnormal T cell membrane function with other discrete T cell dysfunctions (2–15) provides evidence in support of a disorder intrinsic to the T cell. Whether bound autoantibody could produce the capping defect remains to be established. Notwithstanding, altered biochemical pathways could result in multiple, faulty T cell functions manifested, in part, by anomalous receptor regulation and impaired immunoregulation in SLE.

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