4), which is regarded as a probable sustained effect after a lag required for activation of protein synthesis. The enhanced sodium-potassium adenosine triphosphatase mechanism or "sodium pump" (28) is regarded as a major final pathway of energy expenditure.

The binding of thyroid hormone by the proteins of the cytosol differs strikingly from postulated models for steroid hormone action wherein the cytosol receptor-hormone complex is "translocated" into the nucleus, apparently to stimulate the transcription of specific parts of the genetic message. In contrast, the specific T_3 binding sites on cytosol proteins, which number more than 20 million per rat liver cell, may simply hold the hormone inside the cell, facilitating interaction between a minute moiety of intracellular free T_3 and the effector loci (29).

The model we propose consists of an integrated sequence of actions of thyroid hormone at the cellular level, including direct effects on the mitochondria in addition to stimulation of nucleic acid and protein synthesis by way of receptors in the nuclear chromatin. It does not exclude further possible routes of thyroid hormone action, including effects on the plasma membrane (30, 31), and incorporation of the hormone molecule into tyrosine sites in proteins (32). However, on the basis of the available data, it would be most unlikely that binding of thyroid hormone by receptors in the nuclear chromatin could account for the entire spectrum of biological actions, however important such a mechanism may prove to be in growth and differentiation. The lag time before increased protein synthesis (33) shows that such a pathway must represent a sustained rather than an immediate or initial hormone action. In contrast, the activation of mitochondrial energy metabolism would seem a reasonable candidate for the first effects observable, such as increased oxygen consumption soon after administration of T_3 to thyroidectomized rats or to myxedematous human subjects.

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References and Notes

1. H. H. Samuels, J. S. Tsai, J. Casanova, F. Stanley, *J. Clin. Invest.* **54**, 853 (1974).
2. L. J. DeGroot and J. Torresani, *Endocrinology* **96**, 357 (1975).
3. M. I. Surks, D. H. Koerner, J. H. Oppenheimer, *J. Clin. Invest.* **55**, 50 (1975).
4. K. M. MacLeod and J. D. Baxter, *Biochem. Biophys. Res. Commun.* **62**, 577 (1975).
5. K. Sterling and P. O. Milch, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3225 (1975).
6. K. Sterling, V. F. Saldanha, M. A. Brenner, P. O. Milch, *Nature (London)* **250**, 661 (1974).
7. C. Schnaitman and J. W. Greenawalt, *J. Cell Biol.* **38**, 158 (1968).
8. W. Dillman, M. I. Surks, J. H. Oppenheimer, *Endocrinology* **95**, 492 (1974).
9. V. Herbert *et al.*, *Lancet* **1964-II**, 1017 (1964).
10. S. G. Korenman, *J. Clin. Endocrinol. Metab.* **28**, 127 (1968).
11. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
12. K. Sterling, P. Rosen, M. Tabachnick, *J. Clin. Invest.* **41**, 1021 (1962).
13. K. Sterling, *ibid.* **43**, 1721 (1964).
14. J. T. Edsall and J. Wyman, *Biophysical Chemistry* (Academic Press, New York, 1958), vol. 1, pp. 651–653.
15. P. L. Altman and D. S. Dittmer, *Biology Data Book* (Federation of American Societies for Experimental Biology, Washington, D.C., 1964).
16. P. Berg and D. Boman, *Biochim. Biophys. Acta* **321**, 585 (1973).
17. M. E. Pullman, *Methods Enzymol.* **10**, 57 (1967).
18. The tissues of the adult thyroidectomized rat on replacement therapy show increased oxygen consumption in vitro, with the exception of the brain, spleen, and testis [S. B. Barker and H. M. Klitgaard, *Am. J. Physiol.* **170**, 81 (1952)].
19. L. E. Braverman, S. H. Ingbar, K. Sterling, *J. Clin. Invest.* **49**, 855 (1970).
20. K. Sterling, M. A. Brenner, E. S. Newman, *Science* **169**, 1099 (1970).
21. C. S. Pittman, J. B. Chambers, V. H. Read, *J. Clin. Invest.* **50**, 1187 (1971).
22. J. R. Bronk, *Science* **153**, 638 (1966).
23. B. M. Babior, S. Creagan, S. H. Ingbar, R. S. Kipnes, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 98 (1973).
24. M. P. Primack, D. F. Tapley, J. Buchanan, *Endocrinology* **91**, 840 (1972).
25. J. R. Tata, L. Ernster, E. M. Suranyi, *Biochim. Biophys. Acta* **60**, 461 (1962).
26. F. Hoch, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 506 (1967).
27. J. R. Tata, *Nature (London)* **257**, 18 (1975).
28. Y. Asano, U. A. Liberman, I. S. Edelman, *J. Clin. Invest.* **57**, 368 (1976).
29. K. Sterling, *Bull. N.Y. Acad. Med.* **53**, 260 (1977).
30. I. D. Goldfine, C. G. Simons, S. H. Ingbar, *Endocrinology* **96**, 802 (1975).
31. I. D. Goldfine, C. G. Simons, G. J. Smith, S. H. Ingbar, *ibid.*, p. 1030.
32. M. D. Dratman, *J. Theor. Biol.* **46**, 255 (1974).
33. J. R. Tata and C. C. Widnell, *Biochem. J.* **98**, 604 (1966).
34. We are grateful for the analogs generously supplied by E. C. Jorgensen. Supported in part by PHS grant AM 10739 and by the Veterans Administration Medical Research Service. Send reprint requests to the Protein Research Laboratory, VA Hospital, Bronx, N.Y. 10468.

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Induction of Suppressor T Cells in Systemic Lupus Erythematosus by Thymosin and Cultured Thymic Epithelium

Abstract. *Patients with systemic lupus erythematosus lacked suppressor T cell function. Suppressor cell activity was induced in cells from many of these patients by incubation with thymosin or cultured thymic epithelium. These results suggest that thymic manipulation may be a useful therapeutic modality in this disease.*

Systemic lupus erythematosus (SLE) is a serious autoimmune disease in humans. The reasons for the aberrant immune responses in this disorder are unknown. Several investigators suggest that a loss of immunoregulatory suppressor T cells is important in the pathogenesis of SLE (1, 2). In NZB/NZW mice, an excellent animal model of SLE, Krakauer *et al.* (2) provided direct evidence of loss of suppressor cell function with age. We have investigated suppressor T cell activity in human SLE and report that patients with this disease lack suppressor T cells and that suppressor T cell activity is induced in vitro in cells from these patients by incubation with thymosin or cultured thymic epithelium (CTE).

Suppressor T cell activity was determined by assessing the effect of concanavalin A (Con A)–treated lymphocytes on the one-way mixed leukocyte

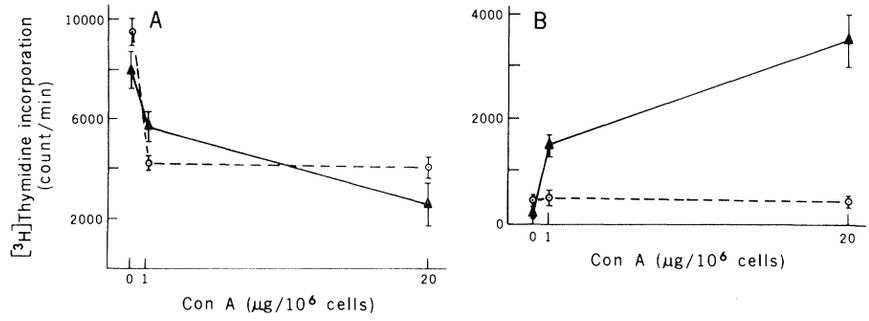

Fig. 1. Assays in normals. (A) The suppressor cell assay was performed as described (3). Representative data from one individual (autologous suppressor assay) are shown; they were similar in all 15 control patients. The allogeneic suppressor assay with normal suppressor cells gave similar results. Each point represents the mean counts per minute ± standard deviation of triplicate determinations. (○) Suppressor cells first incubated with Con A for 24 hours. (▲) Suppressor cells first incubated with Con A for 48 hours. (B) The Con A proliferation assay was performed as described (11). Representative data from one individual are shown but were similar in all 15 control patients. (○) Cells incubated with Con A for 24 hours. (▲) Cells incubated with Con A for 48 hours.

culture (MLC) reaction (*3*). Briefly, peripheral blood mononuclear cells separated on a Ficoll-Hypaque gradient were incubated with Con A (0 to 60 μg/ml) for 24 to 48 hours; the cells were then washed extensively, irradiated, and added to a one-way MLC reaction. The proliferative response was measured by [^{3}H]thymidine incorporation after a 5-day culture period; the percentage of suppression was calculated (Table 1).

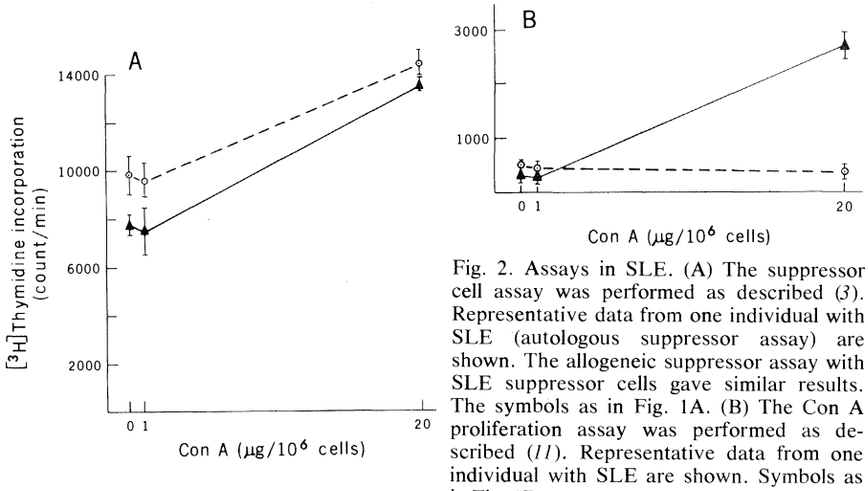

Fig. 2. Assays in SLE. (A) The suppressor cell assay was performed as described (*3*). Representative data from one individual with SLE (autologous suppressor assay) are shown. The allogeneic suppressor assay with SLE suppressor cells gave similar results. The symbols as in Fig. 1A. (B) The Con A proliferation assay was performed as described (*11*). Representative data from one individual with SLE are shown. Symbols as in Fig. 1B.

Patients included in our study satisfied the following criteria for the diagnosis of SLE: typical clinical features, serum antibody to native DNA (titer ≥ 1 : 16), decreased serum complement (C3) during active disease, and characteristic renal biopsy by light and immunofluorescent microscopy (*4*).

In normal individuals (N = 15), significant suppression of the one-way MLC reaction was induced by cells incubated with Con A for 24 or 48 hours (Fig. 1A). Fractionation studies indicated that the suppressor cells were T cells (*5*). Suppression was demonstrated with either autologous or allogeneic suppressor-responder cell combinations. In contrast, SLE patients (N = 14) lacked suppressor cell activity although 13 of 14 had normal Con A–induced proliferation (Fig. 2 and Table 1). Indeed, addition of Con A–activated cells from 9 of 14 lupus patients significantly enhanced the MLC response (Table 1). In patient K.C., sup-

Table 1. Suppressor cell function in SLE. Abbreviations, P, prednisone; A, azathioprine; and C, cyclophosphamide.

Patient	Age (years)	Disease activity*	Therapy			Con A proliferation† (stimulation index)	Suppressor assay‡ (% change)	P§	Suppressor assay			
			Amount (mg)	Drug	Interval (hours)				Thymosin‖ (% change)	P§	CTE‖ (% change)	P§
Females												
T.F.	18	0/5	0	None		14.8	−11	NS	28	<.05	24	<.05
A.W.	18	0/5	0	None			−88	<.01				
		2/5	40	P	24	9.6	12	NS	−14	NS		
A.L.	16	**2**/5	40	P	48	10.7	−11	NS				
		1/5	40	P	48	20.8	9	NS	55	<.05	20	<.01
M.M.	21	0/5	15	P	48							
			100	A	24	13.8	1	NS	48	<.02	47	<.001
K.C.	12	0/5	25	P	24	6.4	−20	<.1			49	<.02
		4/5	25	P	48	7.9	47	<.01	−24	NS	1	NS
		3/5	60	P	48	39.8	−8	NS	7	NS		
T.C.	24	1/4	30	P	48							
			100	A	24	17.0	−121	<.02	62	<.01		
L.P.	46	3/5	30	P	24							
			125	A	24	6.8	−78	<.001	64	<.002		
S.C.	18	0/3	60	P	48							
			75	C	24		−46	<.002				
		1/4	40	P	48	10.7	11	NS	9	<.1	4	NS
J.M.	23	0/5	0	None		11.4	−37	<.01	1	NS	−128	<.001
A.B.	23	4/5	50	P	48							
			100	A	24	29.3	−3	NS	21	<.05		
H.P.	36	2/5	20	P	48							
			50	A	24	10.4	−39	<.1	−15	NS		
K.K.	25	4/5	40	P	48	16.9	−29	<.02			−3	NS
		1/5	40	P	48	23.0	−65	<.02			9	NS
Males												
G.B.	13	**4**/5	60	P	24		−36	<.01				
		3/5	55	P	48	4.3	−167	<.02				
C.T.	14	1/5	55	P	48	13.8	7	NS	16	NS		

*Based on clinical status and laboratory studies—that is, double-stranded DNA antibody, serum complement (C3), sedimentation rate, and urinalysis. The ratio 0/5 means no active clinical disease and normal laboratory parameters; 5/5 means active disease and abnormalities of all four laboratory indicators. Boldface indicates active clinical disease. If the denominator is less than 5, then not all laboratory studies were performed at that particular clinic visit. †Con A proliferation assay performed as described in (*11*). Cells were incubated with Con A for 48 hours. Results are expressed as the stimulation index (counts per minute, experimental/control). A stimulation index of ≥ 5 is normal. ‡Autologous suppressor assay performed as described in (*3*). SLE "suppressor" cells were first incubated with Con A for 48 hours. The results are expressed as the percent change, which is 100 times the ratio of the difference in counts per minute between the control and experimental, to the counts per minute in the control. A positive percent change indicates a suppressor effect, whereas a negative percent change indicates a helper effect. The allogeneic suppressor assay gave similar results. §Differences between means were statistically analyzed with use of the Student's t-test for unpaired variables; NS, not significant. ‖Peripheral blood mononuclear cells (3×10^6) from SLE patients in 1 ml of RPMI 1640 with 15 percent heat-inactivated serum were incubated with medium, 250 μg of calf thymosin fraction 5 or CTE for 1 to 7 days, washed extensively, adjusted to a concentration of 1×10^6 viable cells per milliliter, and irradiated (4000 rads); 1×10^5 irradiated cells in 0.1 ml were then added to an MLC reaction (allogeneic suppressor assay). The data shown are for the cells incubated with thymosin for 48 hours or CTE for 7 days. The results are expressed as the percent change.

pressor function was demonstrated on one of three occasions.

Cells from lupus patients were incubated with calf thymosin fraction 5 (*6*) or CTE (*7*) for 1 to 7 days, washed, irradiated, and added to an MLC reaction (Table 1). After incubation with thymosin (250 μg/ml) for 24 to 48 hours, cells from 7 of 12 patients with SLE significantly suppressed the MLC reaction; in two patients suppressor activity was not induced until cells were exposed to thymosin (500 μg/ml). Culture of SLE cells with CTE for 7 days led to the generation of suppressor cells in four of seven patients; in patient J.M., exposure to CTE led to the induction of a significant helper effect (*8*).

Our results indicate that SLE patients lack suppressor function. We do not feel that the lack of suppressor function in SLE is due to excess helper activity since dilutional studies with Con A–activated lupus cells did not demonstrate suppression of the MLC reaction. Abdou *et al.* (*9*) and Breshnihan and Jasin (*10*), using different assay systems, reported that suppressor function was depressed in SLE, but returned toward normal as the activity of the disease declined. However, in our studies suppressor function was absent in 14 consecutive SLE patients, including three patients with inactive disease who had not received any drug therapy for more than 6 months and three other patients with inactive disease who had been treated with steroids or immunosuppressive agents (or both). We also found that suppressor function could be induced in SLE cells after incubation with thymosin or CTE, suggesting that thymic manipulation may be a useful therapeutic modality in some cases of SLE.

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References and Notes

1. N. Talal, *Arthritis Rheum.* **13**, 887 (1970); N. L. Gerber, J. A. Hardin, T. M. Chused, A. D. Steinberg, *J. Immunol.* **113**, 1618 (1974); A. D. Steinberg, *Arthritis Rheum.* **17**, 11 (1974); N. Talal and A. D. Steinberg, *Curr. Top. Microbiol. Immunol.* **64**, 79 (1974).
2. R. S. Krakauer, T. A. Waldmann, W. Strober, *J. Exp. Med.* **144**, 662 (1976).
3. L. Shou, S. A. Schwartz, R. A. Good, *ibid.* **143**, 1100 (1976). Peripheral blood mononuclear cells (3×10^6), obtained by Ficoll-Hypaque centrifugation, were cultured in 1 ml of medium RPMI 1640 with 15 percent AB serum (inactivated by heat) containing Con A (0 to 60 μg/ml) (0 to 20 μg of Con A per 10^6 cells) for 24 to 48 hours (37°C in a 5 percent CO_2 atmosphere), washed three times, and irradiated (4000 rads). Cells cultured without Con A in the media served as control cells for the suppressor assay. Either 1×10^5 (0.1 ml) of viable Con A–treated, irradiated "suppressor" cells or 1×10^5 (0.1 ml) of viable control irradiated cells were added to a one-way MLC reaction containing 1×10^5 responder cells and 1×10^5 irradiated stimulator cells in medium RPMI 1640 with 20 percent pooled human plasma (inactivated by heat). In the autologous suppressor assay the responder cells were from either the patient or a normal control (A), and the suppressor cells were Con A–treated, irradiated cells from the same individual (A). In the allogeneic suppressor assay the responder cells were from a normal individual (B), and the suppressor cells were from the patient (A) or a different normal individual (A), and Con A–treated (irradiated). In both assays the stimulator cells were irradiated cells from a normal individual (C). After a 5-day culture period, cells were briefly treated with ^{3}H-labeled thymidine (1 μc/0.05 ml) and harvested 16 hours later; radioactivity was counted in a Packard 3375 liquid scintillation spectrometer.
4. A. S. Cohen, W. E. Reynolds, E. C. Franklin, J. P. Kulka, M. W. Ropes, L. E. Shulman, S. L. Wallace, *Bull. Rheum. Dis.* **21**, 643 (1971); D. S. Baldwin, J. Lowenstein, N. F. Rothfield, G. Gallo, R. T. McCluskey, *Ann. Intern. Med.* **73**, 929 (1970).
5. Normal peripheral blood mononuclear cells obtained by Ficoll-Hypaque centrifugation were further fractionated, utilizing adherence to plastic and E-rosette formation. T cells were defined by total E-rosette formation, B cells were defined by surface immunoglobulin detected by direct immunofluorescence, and monocytes were defined by peroxidase staining. A T cell–enriched population (more than 95 percent T cells, less than 1 percent B cells, and less than 1 percent monocytes) incubated with Con A for 48 hours suppressed the MLC reaction ($N = 5$); a monocyte-enriched population (less than 5 percent B cells and less than 3 percent T cells) and a B cell–enriched population (less than 3 percent T cells and less than 2 percent monocytes) incubated with Con A for 48 hours did not suppress the MLC reaction ($N = 4$).
6. Pyrogen-free (clinical quality) calf thymosin fraction 5; J. A. Hooper, M. C. McDaniel, G. B. Thurman, G. H. Cohen, R. S. Schulof, A. L. Goldstein, *Ann. N.Y. Acad. Sci.* **249**, 125 (1975).
7. R. Hong, M. Santosham, H. Schulte-Wissermann, S. Horowitz, S. H. Hsu, J. A. Winkelstein, *Lancet* **1976-II**, 1270 (1976).
8. Human thymic fragments (CTE) were grown for 13 to 24 days prior to incubation with SLE cells. Initial observations suggest that the duration of initial thymic culture affects the ability of CTE to induce suppressor cell function. In patient J.M., the CTE was grown for 13 days prior to incubation with SLE cells; this short culture period may explain the induction of a helper effect.
9. N. I. Abdou, A. Sagawa, E. Pascual, J. Herbert, S. Sadeghee, *Clin. Immunol. Immunopathol.* **6**, 192 (1976).
10. B. Breshnihan and H. E. Jasin, *J. Clin. Invest.* **59**, 106 (1977).
11. Peripheral blood mononuclear cells (1×10^5) were cultured in 0.1 ml of medium RPMI 1640 with 15 percent AB serum (inactivated by heat) containing 0 to 20 μg of Con A per milliliter (0 to 20 μg of Con A per 10^6 cells) for 24 to 48 hours at 37°C, in a 5 percent CO_2 atmosphere. ^{3}H-labeled thymidine was present during the last 16 hours of culture.
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Defective Phagocytosis of Isolated Rod Outer Segments by RCS Rat Retinal Pigment Epithelium in Culture

Abstract. *Retinal pigment epithelium cultured from normal rats phagocytizes large amounts of rod outer segment fragments isolated from normal rats and from RCS rats with inherited retinal degeneration. Cultured RCS rat pigment epithelium rarely ingests outer segment material, although the cells extend cellular processes around fragments of either type. Both normal and RCS pigment epithelium phagocytize polystyrene spheres. This demonstrates that RCS rat pigment epithelial cells contain a defect in the mechanism for phagocytizing outer segments.*

Phagocytosis of the tips of rod outer segments by the retinal pigment epithelium occurs as part of the outer segment renewal process (*1*). A balance is thereby established between the synthesis of new disks at the base of the outer segment (*2*) and removal at the distal ends. In the RCS rat with inherited retinal degeneration, phagocytosis of outer segment tips by the pigment epithelium fails to occur in vivo (*3–5*), resulting in the accumulation of membranous outer segment debris in the subretinal space and degeneration of the photoreceptor cells (*4–6*). Phagocytosis is not totally defective, since RCS rat pigment epithelial cells are capable of ingesting carbon particles injected into the subretinal space (*7*).

The pigment epithelium has been shown to be the primary site of expression of the mutant RCS gene in chimeras formed by combining embryos of a normal and an RCS rat. Accumulation of membranous debris and loss of photoreceptor cells occurred only opposite the RCS pigment epithelium in the chimeras, while photoreceptor cells with outer segments in contact with normal pigment epithelium were unaffected (*8*). However, it has not been demonstrated how the RCS gene is expressed in the pigment epithelium. The RCS pigment epithelial cell may have a defective mechanism for phagocytizing outer segment material, or the RCS pigment epithelium may prevent phagocytosis by adversely influencing outer segment differentiation or altering outer segment membrane properties. In the study described herein we compared the ability of cultured normal and