A Serum Inhibitor of Immune Regulation in Patients with Systemic Lupus Erythematosus

JEREMIAH J. TWOMEY, ARLINE H. LAUGHTER, and ALFRED D. STEINBERG,
*The Veterans Administration Hospital and Baylor College of Medicine,
Houston, Texas 77211 and the Arthritis and Rheumatism Branch,
National Institute of Arthritis, Metabolism, and Digestive Diseases,
National Institutes of Health, Bethesda, Maryland 20014*

ABSTRACT Normal mononuclear leukocytes were incubated with serum from patients with active systemic lupus erythematosus (SLE) and healthy subjects and then studied in lymphoproliferative tests. Serum from SLE patients that contained an autoantibody to a subpopulation of thymus-derived (T) lymphocytes inhibited suppressor T-cell activity induced with concanavalin A. These sera did not inhibit lymphoproliferative responses or suppression by monocytoïd cells. Mitogen-activated suppressor cells were not inhibited with serum from SLE patients or healthy subjects lacking T-cell autoantibody. This abnormality may contribute to the altered immune response that occurs with SLE.

INTRODUCTION

Humoral immunity is abnormally active with systemic lupus erythematosus (SLE).¹ This hyperactivity is evidenced by increased nucleotide synthesis by bone marrow-derived (B) lymphocytes (1), high numbers of circulating lymphocytes producing antibodies (2), and elevated immunoglobulin synthesis (3). A variety of autoantibodies are found with SLE, including antibodies to lymphocytes (4). These autoantibodies may contribute to the pathogenesis of the disease.

Address reprint requests to Dr. Twomey, Veterans Administration Hospital, Houston, Tex. 77211.

Received for publication 17 February 1978 and in revised form 2 June 1978.

¹Abbreviations used in this paper: MLR, mixed leukocyte reaction; SLE, systemic lupus erythematosus.

Inbred NZB and NZB/W mice develop autoimmune abnormalities similar to SLE in man. These mice develop a deficit of suppressor thymus-derived (T) cells (5) which may be related to an autoantibody directed against a subpopulation of T cells involved with immune regulation (6).

A similar abnormality may exist with SLE where a deficiency of concanavalin A-induced suppressor T cells (7) and autoantibodies to T lymphocytes have also been reported (8). An anti-T-cell antibody occurs with active SLE which depletes a subpopulation of T cells through antibody-dependent cellular cytotoxicity (9). We wish to introduce evidence that this antibody interferes with immune regulatory mechanisms by inhibiting suppression by T lymphocytes preactivated with concanavalin A (10). This antibody probably contributes to the lymphopenia with active SLE and may cause preferential depletion of cells involved with immune regulation.

METHODS

Heparinized blood was collected from healthy young adults and mononuclear leukocytes harvested by isopycnic flotation (11). Adherent cells were depleted by incubation with glass wool (12). T cells were separated from mononuclear leukocytes by forming E rosettes (13), and B cells by forming EAC rosettes (14), which were then centrifuged through Ficoll-Hypaque (Ficoll, Pharmacia Fine Chemicals, Inc., Piscataway, N. J.; Hypaque, Winthrop Laboratories, New York). Triplicate 0.2-ml mixed leukocyte reaction (MLR) cultures were prepared and response by 10⁵ lymphocytes to 10⁵ irradiated allogeneic lymphocytes was measured by [³H]thymidine incorporation during the 7th day of incubation (15). To test

for suppressor T-cell activity (10), an additional 10^5 non-adherent cells were added to paired MLR cultures after these added cells had been preincubated for 40 h (a) with 60 μ g concanavalin A per 5×10^6 lymphocytes or (b) without concanavalin A. After preincubation with concanavalin A, >90% lymphocytes excluded trypan blue. To test for adherent suppressor cell activity (15), paired MLR cultures were prepared in which stimulation was with (a) 8×10^5 lymphocytes and (b) 2×10^6 lymphocytes from the same fresh mononuclear leukocyte preparations. Percent suppression in both suppressor tests was determined from the formula $(1 - [a \div b]) \times 100$. The effect of heat-decomplemented sera upon mononuclear leukocyte function was tested by preincubation for 30 min at 37°C at 10^7 lymphocytes per milliliter of serum, after which the cells were washed with Hanks' balanced salt solution before being added to cultures. Different leukocyte preparations were used and preincubations with patient and normal sera were conducted in double-blind fashion.

Antibodies to T cells were detected as previously described (9) and further studied by immunofluorescence. Test serum was reacted with normal cells (50μ l/2 $\times 10^6$ cells) for 60 min at 4°C and with fluorescein-conjugated anti-human immunoglobulin that had been centrifuged at 105,000 g for an additional 60 min. Control sera included normal serum and patient serum depleted of antibody by adsorption with leukocytes from a patient with Sézary syndrome. The distribution and intensity of fluorescence was analyzed with a fluorescence-activated cell sorter. Positive patient sera gave significantly more fluorescence than did the control sera. Antibodies that were adsorbed with Sézary cells were designated anti-T-cell antibodies and antibodies that were adsorbed with EAC rosetting cells were designated anti-B-cell antibodies.

Antibodies reactive with native DNA were assayed by a modified Farr assay with 14 C-native human DNA (14); this DNA contained <10% single-stranded molecules. Antiglobulins were measured by bentonite flocculation.

RESULTS

In 50 control experiments without pretreating test cells with serum, mean $\pm$ SEM suppression of MLR responses by concanavalin A-preincubated mononuclear leukocytes was $61 \pm 3\%$. Similar values ($65 \pm 5\%$ suppression) were recorded after mononuclear cells had been preincubated with 10 normal sera (Table I). Suppression was also unaltered after preincubation with sera from seven patients with SLE whose autoantibody responses did not include T-cell autoantibodies. In contrast, suppression was significantly less after preincubation with serum from eight patients with active SLE that did contain antibody to the D subpopulation of T cells (9) ($P < 0.001$, Student *t* test). Inhibition of suppressor T cells was weak with sera that contained low titers of this antibody. Similar results were obtained when three of these sera were retested with different leukocyte preparations.

Normal mononuclear leukocytes were preincubated with the above sera, washed and then tested for adherent suppressor cell activity in the MLR (15). The effect of preincubation with test sera upon lymphoproliferative responsiveness in the MLR and to phytohemagglutinin was also evaluated. Neither adherent

TABLE I
Antibody That Inhibits Suppressor T Cells with SLE

Autoantibody reactivity	[³ H]Thymidine incorporated		Suppression
	Without concanavalin A	With concanavalin A	
		cpm	%
Group A (SLE)			
T-cell subpopulation	48,800	48,400	2
	107,900	77,800	20, 1*
	134,500	108,100	20, 36*
	151,500	104,900	31, 12*
	31,900	21,000	33
Low titer	143,800	95,900	34
Low titer	21,200	13,600	36
Low titer	52,600	33,400	37
Group B (SLE)			
B cells	69,500	11,400	86
B cells	40,600	5,800	86
DNA	29,300	9,600	76
Nuclear antigens	108,600	23,100	76
Antiglobulins	41,200	11,500	72
None	67,600	23,400	65
Group C (Healthy controls)			
10 subjects, mean ± SEM			65 ± 5

The effect of preincubation with SLE sera upon concanavalin-A-induced suppression of the mixed leukocyte reaction. Different leukocyte preparations were used in most experiments.

* Repeat studies with different mononuclear leukocyte donors.

cell suppressor activity nor lymphoproliferative responsiveness was lowered by preincubation with test sera.

DISCUSSION

Normal human leukocytes contain at least two distinct suppressor systems. In one, suppression is brought about by a monocytoïd cell, is determined by cell concentration, and does not require preactivation (15). In the other, the suppressor cell is a nonadherent T lymphocyte that requires preactivation with mitogen (10). The present study indicates that SLE serum which contains an antibody to a subpopulation of T cells (9) inhibits the latter suppressor system. Because this effect is lost after repeated freezing and thawing, inhibition would probably have been greater if the sera had been tested before storage at -70°C . It cannot also be stated with certainty whether the T-cell antibody or concomitantly present immune complexes (which are likely to be disassociated by repeated freezing and thawing) are directly responsible for the phenomenon.

Normal suppressor T-cell activity is lost after leuko-

cytes from healthy subjects are cultured for a number of days (16). This loss of regulatory control is manifested by an increase in lymphoproliferative responses to concanavalin A (17) and the appearance of an MLR response between autologous cells (18) after leukocytes are preincubated for 1 or more days. Fresh leukocytes from patients with SLE lack these manifestations of suppressor cell activity (16–19). Such a deficit of immune regulation could have a central role in the pathophysiology of SLE. Our observation that SLE serum interferes with suppressor T-cell function suggests a mechanism whereby immunologic homeostasis could be impaired in at least some patients with SLE. The underlying abnormality responsible for this T-cell autoantibody remains to be determined.

The present study suggests that suppressor T cells activated by concanavalin A have physiologic importance. The suppressor system effected through monocytoid cells was not appreciably affected by the autoantibody under study and may be intact with SLE. If so, then it is insufficient by itself to maintain normal immunoregulatory control.

ACKNOWLEDGMENT

This research was supported by grant RO1 CA 16108-04, awarded by the National Cancer Institute, Department of Health, Education, and Welfare.

REFERENCES

1. Glinski, W., M. E. Gershwin, D. R. Budman, and A. D. Steinberg. 1976. Study of lymphocyte subpopulations in normal humans and patients with systemic lupus erythematosus by fraction of peripheral blood lymphocytes on a discontinuous Ficoll gradient. *Clin. Exp. Immunol.* **26**: 228–238.
2. Budman, D. R., E. B. Merchant, A. D. Steinberg, B. Dof, M. E. Gershwin, E. Lizzio, and J. P. Reeves. 1977. *Arthritis Rheum.* **20**: 829–833.
3. Jasin, H. E., and M. Ziff. 1975. Immunoglobulin synthesis by peripheral blood cells in systemic lupus erythematosus. *Arthritis Rheum.* **18**: 219–228.
4. Stastny, P., and M. Ziff. 1971. Antibodies against cell membrane constituents in systemic lupus erythematosus and related diseases. *Clin. Exp. Immunol.* **8**: 543–550.
5. Gerber, N. L., J. A. Hardin, T. M. Chused, and A. D. Steinberg. 1974. Loss with age in NZB/W mice of thymic suppressor cells in the graft-vs-host reaction. *J. Immunol.* **113**: 1618–1625.
6. Klassen, L. W., R. S. Krakaner, and A. D. Steinberg. 1977. Selective loss of suppressor cell function in New Zealand mice induced by NTA. *J. Immunol.* **119**: 830–837.
7. Horowitz, S., W. Bordierding, A. Vishnu Moorthy, R. Chesney, H. Schulte-Wisserman, and R. Hong. 1977. Induction of suppressor T cells in systemic lupus erythematosus by thymosin and cultured thymic epithelium. *Science (Wash. D. C.)*. **197**: 999–1001.
8. Lies, R. B., R. P. Messner, and R. C. Williams. 1973. Relative T cell specificity of lymphocytotoxins from patients with systemic lupus erythematosus. *Arthritis Rheum.* **16**: 369–375.
9. Glinski, W., M. E. Gershwin, and A. D. Steinberg. 1976. Fractionation of cells on a discontinuous Ficoll gradient. *J. Clin. Invest.* **57**: 604–614.
10. Shou, L., S. A. Schwartz, and R. A. Good. 1976. Suppressor cell activity after concanavalin A treatment of lymphocytes from normal donors. *J. Exp. Med.* **143**: 1100–1110.
11. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest. Suppl.* **97**: 77–89.
12. Folch, H., and B. H. Waksman. 1974. The splenic suppressor cell. II. Suppression of the mixed leukocyte reaction by thymus-dependent adherent cells. *J. Immunol.* **113**: 140–144.
13. Gershwin, M. E., W. Glinski, T. Chused, and A. D. Steinberg. 1977. Lymphocyte-dependent antibody-mediated cytolysis of DNA-anti-DNA-coated target cells using human and murine SLE effect of populations. *Clin. Immunol. Immunopathol.* **8**: 280–291.
14. Steinberg, A. D., T. Pincus, and N. Talal. 1969. DNA binding assay for detection of anti-DNA antibodies in NAB/NZW F₁ mice. *J. Immunol.* **102**: 788–791.
15. Laughter, A. H., and J. J. Twomey. 1977. Suppression of lymphoproliferation by high concentrations of normal human mononuclear leukocytes. *J. Immunol.* **119**: 173–179.
16. Stobo, J. D. 1977. Immunosuppression in man: Suppression by macrophages can be mediated by interactions with regulatory T cells. *J. Immunol.* **119**: 918–924.
17. Bresnihan, B., and H. E. Jasin. 1977. Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosus. *J. Clin. Invest.* **59**: 106–116.
18. Stobo, J. D., and C. P. Loehnen. 1976. Immunoregulation and autoimmunity. *Mayo Clin. Proc.* **51**: 479–483.
19. Abdou, N. I., A. Sagaiva, E. Pascual, J. Hebert, and S. Sadegher. 1976. Suppressor T-cell abnormality in idiopathic systemic lupus erythematosus. *Clin. Immunol. Immunopathol.* **6**: 192–199.