Clin. exp. Immunol. (1974) **18,** 295–301.

IMPAIRED RESPONSIVENESS OF LYMPHOCYTES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

NICOLE SUCIU-FOCA, J. A. BUDA, TRAUTE THIEM AND K. REEMTSMA

Department of Surgery, College of Physicians and Surgeons, Columbia University, New York City, New York, U.S.A.

(*Received* 6 *February* 1974)

SUMMARY

Cellular immune responsiveness, as measured by lymphocyte transformation in one-way mixed leucocyte cultures (MLC) and in phytohaemagglutinin (PHA) stimulated cultures was evaluated in forty patients with systemic lupus erythematosus (SLE) and in seventy-four normal controls. The effect produced by sera from these subjects on *in vitro* lymphocyte reactivity was tested on autologous cells and on homologous responding cells from a constant panel of ten healthy volunteers.

The reactivity of lymphocytes from SLE patients to PHA and to a battery of allogeneic cells was significantly lower than that of normal controls.

Sera from some SLE patients inhibited the MLC reactions, while in other cases a distinct stimulatory effect was found.

It is suggested that virus-induced modifications of normal histocompatibility antigens cause the appearance of blocking antibody that might bind to the surface of T lymphocytes, impairing their function.

INTRODUCTION

The occurrence in systemic lupus erythematosus (SLE) of immunological abnormalities such as auto-antibodies, skin reactivity to autologous cell constituents and decreased serum complement levels, together with the various analogies with experimentally induced immunological diseases, have led to the view that SLE represents an auto-immune disease (Holman, 1971).

Since thymus-derived (T) lymphocytes seem to play a major role in preventing auto-immunity, one might suspect impairment of T-lymphocyte function to be primarily responsible for the development of auto-antibodies or of diseases such as SLE (Allison, Denman & Barnes, 1971; Fudenberg, 1971; Holman, 1971; Katz & Benacerraf, 1972).

We have investigated the cellular immune competence of patients with SLE and of control subjects, using as functional markers for T cells (Good, 1972) the ability of blood lympho-

Correspondence: Dr Nicole Suciu-Foca, Institute of Cancer Research, Francis Delafield Hospital, 99 Fort Washington Avenue, New York, New York 10032, U.S.A.

cytes to respond to *in vitro* stimulation with allogeneic cells (in the mixed leucocyte culture test) and with phytohaemagglutinin (PHA).

Recent studies show that blocking antibody account for modulation of cell-mediated immunity and that such factors can be detected by inhibition of mixed leucocyte culture (MLC) reaction (Buckley, Schiff & Amos, 1972; Ceppellini *et al.*, 1971; Gatti, Yunis & Good, 1973; Hattler, Karesh & Miller, 1971; Sengar, Opelz & Terasaki, 1973; Suciu-Foca *et al.*, 1973, 1974).

In view of these findings, investigations were designed to test the effect of sera from patients with SLE on the MLC reactivity of autologous and normal homologous lymphocytes.

MATERIALS AND METHODS

Evaluation of lymphocyte responsiveness

Lymphocyte responsiveness was investigated in forty patients with SLE and in seventy-four age-matched normal controls. The patients fell into the following age groups: three were younger than 20 years; thirty-one were between the ages of 21 and 40 years; six patients were 55–70 years of age. The age distribution of the controls was the same as that of the patients, with two controls for each patient in all but six subjects from the middle age group.

Each subject's lymphocytes were tested simultaneously for PHA and MLC reactivities in the presence of autologous serum and of homologous pooled sera from a healthy volunteer panel. A previously described microculture system was used for these studies (Suciu-Foca *et al.*, 1973).

Lymphocyte cultures

Lymphocyte suspensions were prepared from heparinized blood by Ficoll–Isopaque gradient method. All cells were washed three times and grown in RPMI-1640 medium supplemented with glutamine, penicillin and streptomycin. Lymphocytes were distributed into microtitre plates in a total volume of 0·2 ml of medium, to which 0·05 ml of serum was added just before the start of incubation.

The cultures were incubated in a 5% CO_2 atmosphere at 37°C for 5 days, then labelled by addition of 1 μCi of tritiated thymidine per well and harvested with a Multiple Automated Sample Harvester. Label incorporation was measured by liquid scintillation counting in a Packard 3375 Spectrometer. Differences in counts per minute (ct/min) between replicate cultures were less than 10%.

MLC test

Responding lymphocytes were cultured in MLC with mitomycin-treated allogeneic lymphocytes (stimulating cells) obtained from a selected panel of ten unrelated donors. The HL-A antigens covered by this selected panel were the following: HL-A 1, 2, 3, 9, 10, 11, W19, W28 (of the first locus) and HL-A 5, 7, 8, 12, 13, W5, W10, W15, W17 (of the second locus).

Quadruplicate cultures of responding cells were run against a standard stimulating cell mixture made up of equal amounts of cells from the ten donors. Each culture contained 1×10^5 responding cells and 2×6^5 stimulating cells. At the end of the incubation time viability was determined by Trypan Blue exclusion test using the fourth culture of the

quadruplicates. Mean ct/min of the remaining triplicate cultures were calculated for each of the responders, and used for expressing the MLC reactivity (MLCR).

PHA stimulation test

Triplicate cultures of responding cells, containing 2×10^5 lymphocytes per well were grown in the presence of purified PHA (Burroughs Wellcome Phytomitogen MR 68) at a final concentration of 5·0 μg/ml. PHA reactivity (PHAR) was expressed as mean ct/min in triplicate cultures.

Screening of inhibitory serum factors

Responding lymphocytes from ten healthy staff members were exposed to the standard stimulating cell mixture in the presence of sera from forty SLE patients and fifty normal controls. Parallel MLC were grown with pooled normal sera.

Mean ct/min in triplicate cultures were calculated for each of the responders, and all of the ten values obtained for one serum were included in the determination of the effect of the serum on the MLCR of the responding cell panel.

The ratio MLCR in the tested serum over MLCR in pooled normal sera was calculated for each serum and used for expressing its effect on the responding cell panel. Values lower than 0·50 were considered indicative of inhibitory effect; values in the range of 0·50–1·5 expressed the absence of blocking activity, and values higher than 1·50 were considered indicative of stimulatory serum effect.

RESULTS

MLC and PHA reactivity of lymphocytes from patients with SLE

Table 1 presents a comparison between the lymphocyte reactivity of forty patients with SLE and seventy-four healthy controls. The mean MLC and PHA responsiveness of the patient group was significantly lower ($P > 0{\cdot}001$) than that of the control group, both when autologous and when normal homologous sera were used for the reaction. None of the patients was treated with cytotoxic drugs but twenty-one of them were undergoing corticosteroid therapy. The reactivity of patients receiving steroids did not differ significantly from that of untreated patients, in either MLC or PHA tests.

The effect of each serum on MLCR and PHAR of autologous lymphocytes was expressed by the ratio: reactivity in autologous serum/reactivity in pooled homologous sera.

Lymphocyte reactivity in controls stayed at the same level when tested in autologous or homologous sera. Similarly, lymphocyte reactivity in the majority of SLE patients did not differ significantly when tested in autologous or homologous serum.

In some patients, however, MLCR and PHAR were lower in autologous than in homologous serum (ratio less than 0·5), while in other patients autologous serum enhanced the reactivity of lymphocytes above the levels seen in pooled normal sera (ratio more than 1·5).

Effect of serum from SLE patients on MLC reactivities of autologous and homologous lymphocytes

The effect of sera from forty SLE patients and from fifty healthy controls on the MLC reactivity of autologous and homologous panel lymphocytes is analysed in Table 2.

TABLE 1. MLC and PHA responsiveness* of lymphocytes from patients with SLE and from normal subjects

Subjects	Number of cases	ct/min ±s.e. MLC responsiveness: Autologous serum	MLC responsiveness: Homologous serum	PHA responsiveness: Autologous serum	PHA responsiveness: Homologous serum	Effect of autologous serum† on: MLCR (number of sera showing inhibition/stimulation/no changes)	Effect of autologous serum† on: PHAR (number of sera showing inhibition/stimulation/no changes)
SLE	40	15829±282	18663±250	14932±251	13701±206	8/7/26	5/6/30
Normals	74	34587±104	34820±168	28675±106	27510±125	0/0/74	0/0/74

* Responsiveness is expressed as tritiated thymidine incorporation in triplicate cultures of lymphocytes from each subject included in the group.

† The effect of autologous serum on MLCR and PHAR was expressed by the ratio of reactivity in autologous serum : reactivity in pooled homologous sera. A ratio of less than 0·5 indicates inhibition; 0·5–1·5, no effect; more than 1·5 indicates stimulation.

TABLE 2. Effect of sera from SLE patients on MLCR of autologous and homologous panel lymphocytes

Subjects	MLCR (ct/min) in autologous serum	Total number of subjects	Effect of sera on MLCR of: Autologous lymphocytes: Inhibition	Autologous lymphocytes: Stimulation	Autologous lymphocytes: No changes	Homologous lymphocytes: Inhibition	Homologous lymphocytes: Stimulation	Homologous lymphocytes: No changes
SLE	<10,000	17	8	2	7	5	2	10
patients	10,000–20,000	13		1	12	—	1	12
	>20,000	10		4	6		4	6
Normals	>20,000	50			50			50

Patients were arbitrarily grouped in three categories according to the magnitude of their MLC responsiveness in autologous serum.

Responsiveness, as expressed by tritiated thymidine incorporation in triplicate cultures of lymphocytes (mean ct/min) was below 10,000 ct/min (mean 6531 ± 2829 s.d.) in seventeen patients (very poor responders); in the range of 11,000–20,000 ct/min (mean 15731 ± 2434 s.d.) in thirteen patients (poor responders); and higher than 20,000 ct/min (mean 32,060 ± 7401 s.d.) in ten patients (good responders), as well as in the fifty healthy controls (mean 34,280 ± 4020 s.d.).

Serum inhibitory factors were detected in eight patients from the very low responder group by testing the effect of the sera on autologous lymphocytes; only five of these sera also inhibited the MLCR of panel lymphocytes.

No MLC blocking activity was found in the sera of SLE patients with higher levels of MLCR either when sera were screened in the autologous or in the homologous system.

Sera from seven patients, four of which belonged to the good responder group, enhanced the MLCR of both autologous and homologous panel lymphocytes more than 50% above the level seen when cells were tested in pooled normal serum.

None of the fifty control sera displayed inhibitory or stimulatory activities in either the autologous or homologous screening system.

DISCUSSION

Our observation that lymphocytes from patients with SLE display defective MLCR and PHAR appears to indicate the occurrence of impairment of the cell-mediated arm of the immune response in this disease.

Apparent cellular deficiency or abnormal behaviour of lymphocytes in MLC have been previously described in patients with rheumatoid arthritis and in a limited number of patients with SLE (Astorga & Williams, 1969; Hedberg *et al.*, 1971; Lueker & Williams, 1972; Paty *et al.*, 1974; Wernet *et al.*, 1973).

Determination of the MLCR of different individuals to a randomly selected unrelated subject—as performed in the classical one-way MLC test—does not allow, however, a comparative evaluation of cellular reactivity, since the magnitude of the MLC response is directly related to the antigenic disparity between the responding and the stimulating cell population (Bach & Bach, 1972). In order to eliminate this variable we have tested each individual's MLCR to a standard battery of stimulating allogeneic cells selected to include the major HL-A specificities.

The data obtained in SLE patients are reminiscent of the impaired lymphocyte responsiveness which has been previously documented by us in patients with cancer (Suciu-Foca *et al.*, 1973). We have shown that the deficient lymphocyte response in cancer is at least partially caused by serum blocking factors (Suciu-Foca *et al.*, 1973). Such factors might be antitumoral antibodies with broad cross-reacting HL-A antibody activity which may block the antigens and/or the closely linked immune receptors on the surface of T-lymphocytes (Ceppellini *et al.*, 1971; Suciu-Foca *et al.*, 1973, 1974).

Since viral infections may be involved in the pathogenesis of SLE (Roy *et al.*, 1973) one can postulate that in this disease, too, T-lymphocyte hyporeactivity is caused by antibodies with MLC-blocking and anti-HL-A activities, which have developed in response to virus-induced modifications of normal isoantigens.

This hypothesis is consistent with the finding of lymphocytotoxins which showed apparent T-cell specificity and of IgG antibody which showed MLC blocking activity, in some SLE sera (Williams, Lies & Messner, 1973). Furthermore, such anti-T-cell antibodies seem to be responsible for the lymphocytopaenia, which has been documented in some SLE patients (Messner, Lindström & Williams, 1973; Williams *et al.*, 1973).

A shift in the proportion of T- and B-cell populations may well explain the hyporeactivity of lymphocytes from patients with SLE when tested for T-cell function.

In the present investigation, the strong MLC inhibitory activity displayed on autologous lymphocytes by sera from eight out of the seventeen patients with very low lymphocyte reactivity might also be ascribed to blocking antibodies.

However, only five of these sera were also inhibitory in the homologous system and most of the sera from SLE patients with cellular hyporesponsiveness showed no inhibitory activity at all.

These observations do not exclude, however, the involvement of blocking antibodies which are attached to the receptors of the patient's lymphocytes but whose titre in the serum is too low for being detectable in the homologous system.

This possibility is supported by recent observations on the bidirectional non-reactivity of lymphocytes from patients with various immune deficiencies—when tested in one-way MLC (Astorga & Williams, 1969; Hedberg *et al.*, 1971; Lueker & Williams, 1972)–as well as by the finding that lymphocytes from SLE patients recover their MLCR following preincubation and washing (Wernet *et al.*, 1973).

In this same line of reasoning the rather surprising ability of 15% of the tested sera (seven out of forty) to stimulate MLCR might be attributed to circulating antigen–antibody complexes (Sell & Gell, 1965).

It is possible that deficiency of cellular immunity is a corollary rather than a pathogenetic factor in SLE. Immunodeficiency may result from cytopathic effect of virus with simple reduction in the number of immunocompetent cells or from impairment of intracellular synthetic processes (Feldman, 1972; Klippel, Grimley & Decker, 1974).

In the light of current theory on co-operative interaction between T cells and antibody-forming B cells (Allison *et al.*, 1971; Fudenberg, 1971; Katz & Benacerraf, 1972) one might, however, conceive that sensitization of cells to virus-induced alteration of self-antigens (Lawrence, 1972) can stimulate B cells to produce auto-antibodies, which in turn may suppress the T-cell immune receptors. More studies are required for elucidating the mechanism involved in the impaired cellular reactivity of patients with SLE and for understanding its relationship to the development of the disease.

REFERENCES

ALLISON, A.C., DENMAN, A.M. & BARNES, R.D. (1971) Cooperating and controlling functions of thymus-derived lymphocytes in relation to autoimmunity. *Lancet*, **ii**, 135.

ASTORGA, G.P. & WILLIAMS, R.C. (1969) Altered reactivity in mixed lymphocyte culture of lymphocytes from patients with rheumatoid arthritis. *Arthr. and Rheum.* **12**, 547.

BACH, F.H. & BACH, L.M. (1972) Fundamental immunogenetics—their application to histocompatibility. *Clinical Immunobiology* (ed. by F. H. Bach & R. A. Good), volume 1, p. 157. Academic Press, New York.

BUCKLEY, R.H., SCHIFF, R.I. & AMOS, D B. (1972) Blocking of autologous and homologous leukocyte responses by human alloimmune plasma: a possible *in vitro* correlate of enhancement. *J. Immunol.* **108**, 34.

CEPPELLINI, R., BONNARD, G.D., COPPO, F., MIGGIANO, V.C., POSPISIL, M., CURTONI, E.S., & PELLEGRINO, M. (1971) Mixed leukocyte cultures and HL-A antigens. I. Reactivity of young fetuses, newborns, and mothers at delivery. II. Inhibition by anti-HL-A sera. *Transplant. Proc.* **3,** 58.

FELDMAN, J.D. (1972) Immunological enhancement: a study of blocking antibodies. *Advances in Immunology* (ed. by F. J. Dixon & H. Kunkel), volume 15, p. 167. Academic Press, New York.

FUDENBERG, H.H. (1971) Are autoimmune diseases immunologic deficiency states? *Immunobiology* (ed. by R. A. Good & D. W. Fisher), p. 175. Sinauer Associates, Stamford, Connecticut.

GATTI, R.A., YUNIS, E.J., & GOOD, R.A. (1973) Characterization of a serum inhibitor of MLC reactions. *Clin. exp. Immunol.* **13,** 427.

GOOD, R.A. (1972) Structure–function relations in the lymphoid system. *Clinical Immunobiology* (ed. by F. H. Bach & R. A. Good), volume 1, p. 1. Academic Press, New York.

HATTLER, B.G., JR, KARESH, C. & MILLER, J. (1971) Inhibition of the mixed lymphocyte culture response by antibody following successful human renal transplantation. *Tissue Antigens* (ed. by F. Kissmeyer-Nielsen), volume 1, p. 270, Munksgaard, Copenhagen.

HEDBERG, H., KÄLLÉN, B., LÖW, B. & NILSSON, O. (1971) Impaired mixed leukocyte reaction in some different diseases, notably multiple sclerosis and various arthritides. *Clin. exp. Immunol.* **9,** 201.

HOLMAN, H.R. (1971) Systemic lupus erythematosus. *Immunological Diseases,* 2nd edn (ed. by Max Samter), volume 2, p. 995. Little, Brown and Company, Boston.

KATZ, D.H., & BENACERRAF, B. (1972) The regulatory influence of activated T-cells on B-cell response to antigen. *Advances in Immunology* (ed. by F. J. Dixon & Henry G. Kunkel), p. 1. Academic Press, New York.

KLIPPEL, J.H., GRIMLEY, P.M., & DECKER, J.L. (1974) Lymphocyte inclusions in newborns of mothers with systemic lupus erythematosus. *New Engl. J. Med.* **290,** 96.

LAWRENCE, H.S. (1972) Cellular immunity. *Clinical Immunobiology* (ed. by F. H. Bach and R. A. Good). volume 1, p. 47. Academic Press, New York.

LUEKER, R.D. & WILLIAMS, R.C., JR (1972) Decreased reactivity of lymphocytes in mixed-leukocyte culture from patients with rheumatic fever. *Circulation,* **XLVI,** 655.

MESSNER, R.P., LINDSTRÖM, F.D. & WILLIAMS, R.C. (1973) Peripheral blood lymphocyte cell surface markers during the course of systemic lupus erythematosus. *J. clin. Invest.* **52,** 3046.

PATY, J., SIENKNECHT, C., TOWNES, A., HANISSIAN, A. & MASI, A. (1974) Impaired cell-mediated immunity in systemic lupus erythematosus (SLE). *Clin. Res.* **22,** 30A.

ROY, L.P., FISH, A.J., MICHAEL, A.F. & VERNIER, R.L. (1972) Etiologic agents of immune deposit disease. *Progress in Clinical Immunology* (ed. by R. S. Schwartz), volume 1, p. 1. Grune & Stratton, New York.

SELL, S. & GELL, P.G.H. (1965) Studies on rabbit lymphocytes *in vitro*. I. Stimulation of blast transformation with an antiallotype serum. *J. exp. Med.* **122,** 423.

SENGAR, D.P.S., OPELZ, G. & TERASAKI, P.I. (1973) Outcome of kidney transplants and suppression of mixed leukocyte culture by plasma. *Transplant. Proc.* **5,** 641.

SUCIU-FOCA, N., BUDA, J., MCMANUS, J., THIEM, T. & REEMTSMA, K. (1973) Impaired responsiveness of lymphocyte and serum-inhibitory factors in patients with cancer. *Cancer Res.* **33,** 2373.

SUCIU-FOCA, N., BUDA, J., THIEM, S. & REEMTSMA, K. (1974) Evaluation of the immune status of transplant recipients by mixed lymphocyte culture. *Clin. Immunol. Immunopathol.* (In press.)

WERNET, P., FOTINO, M., THOBURN, R., MOORE, A. & KUNKEL, H.G. (1973) Blockage of lymphocyte surface antigens and the shedding phenomenon in systemic lupus erythematosus (SLE). *Arthr. and Rheum.* **16,** 137.

WILLIAMS, R.C., DEBOARD, J.R., MELLBYE, O.J., MESSNER, R.P., LINSTRÖM. F.D. (1973) Studies of T- and B-lymphocytes in patients with connective tissue diseases. *J. clin. Invest.* **52,** 283.

WILLIAMS, R.C., LIES, R.B. & MESSNER, R.P. (1973) Inhibition of mixed leukocyte responses by serum and gamma-globulin fractions from certain patients with connective tissue disorders. *Arthr. and Rheum.* **16,** 597.