

The Cellular Basis of the Impaired Autologous Mixed Lymphocyte Reaction in Patients with Systemic Lupus Erythematosus

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ABSTRACT The autologous mixed lymphocyte reaction is impaired in patients with systemic lupus erythematosus. This is because nonthymus-derived (T)-lymphocyte preparations from such patients do not stimulate autologous T-lymphocyte proliferation normally. This defect may explain the impaired generation of suppressor activity in systemic lupus erythematosus and thereby the occurrence of autoantibodies in this disease.

INTRODUCTION

A subpopulation of human bone marrow-derived (B) lymphocytes stimulates the proliferation of autologous thymus-derived (T) lymphocytes (1, 2). This autologous mixed lymphocyte reaction (MLR)¹ has two cardinal characteristics of an immune response: immunological memory and specificity (3). The autologous MLR has been shown to provide a proliferative stimulus required for the generation of cytotoxic lymphocytes (4), and preliminary evidence suggests that suppressor activity is generated during this reaction (unpublished observation).

Recently, Sakane et al. (5) reported that lymphocytes from patients with systemic lupus erythematosus (SLE) fail to express an autologous MLR. These authors offered circumstantial evidence that defective T-cell function leads to the impaired autologous MLR. We also had found that the autologous MLR is depressed in patients with SLE (6). We now present evidence that this defect results from a change in the B-cell preparations from patients with SLE. This conclusion was based upon data obtained from three pairs of MLR-identical siblings discordant for SLE.

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

METHODS

Patients studied. 10 patients with SLE were studied. The patients were females between the ages of 19 and 43. The patients were studied when disease activity was minimal. Eight patients were untreated at the time of study; one patient was receiving 10 mg of prednisone per day, and another was receiving 15 mg of prednisone on alternate days. Seven age-matched healthy normal subjects and three normal MLR identical siblings served as controls.

Autologous MLR cultures. The autologous and allogeneic MLR cultures were established as described (1). Briefly, human blood T lymphocytes that formed rosettes with sheep erythrocytes were separated from non-T lymphocytes, referred to in this paper as B lymphocytes. 7% of the T-lymphocyte preparation and 93–98% of the B-lymphocyte preparation reacted with fluoresceinated anti-human immunoglobulin. 15–35% of the mononuclear cells in the B-lymphocyte preparation phagocytized latex particles. Because mononuclear cells can inhibit lymphocyte proliferation, the mononuclear cell suspension was passed over a nylon fiber column to deplete the preparation of adherent cells. After nylon passage, 2–5% of the mononuclear cells were monocytes based upon a histochemical reaction for peroxidase (7). 100,000 purified T lymphocytes were cultured with $1-2 \times 10^5$ -irradiated (3,000 R) autologous or allogeneic B lymphocytes in 0.2 ml RPMI 1640 with 20% human serum from AB type blood donors. Cultures were incubated for 6 d. Thymidine incorporation was measured during the last 24 h of incubation. The average of the counts per minute of triplicate cultures is given.

RESULTS

Autologous and allogeneic MLR in SLE patients. T lymphocytes from normal subjects or patients with SLE were cultured with irradiated autologous or allogeneic B lymphocytes (Table I). The autologous MLR in patients with SLE was strikingly and significantly ($P < 0.003$) depressed compared to the autologous MLR in normal subjects. This was not attributable to differences in the background incorporation of thymidine by T lymphocytes. The impaired autologous MLR was not related to duration of disease or steroid therapy. In contrast to the depressed autologous MLR,

TABLE I
Thymidine Incorporated by T Lymphocytes from Normal Subjects or Patients with SLE Stimulated by B Lymphocytes

Responder cell donor	Stimulating lymphocytes	
	Autologous B lymphocytes	Allogeneic B lymphocytes
	<i>Thymidine incorporated/culture</i> × 10 ⁻³	
Normal (n = 7)	19.9 ± 3.8	35.3 ± 8.5
SLE (n = 7)	3.5 ± 1.1	79.7 ± 11.1

* 10⁵ T lymphocytes were incubated with 2 × 10⁵-irradiated autologous or allogeneic B lymphocytes in 0.2 ml RPMI 1640 with 20% human serum for 6 d. Tritiated thymidin incorporation was measured during the last 24 h of incubation. Counts per minute given are the mean of triplicate cultures. The background counts per minute (T lymphocytes cultured with irradiated autologous T-lymphocytes) averaged 787 cpm for SLE patients and 1,491 for normal subjects. Mononuclear cell suspensions were passed over nylon fiber columns before fractionation into T and B preparations. The data given are the mean ± SE of the mean.

the allogeneic MLR in patients with SLE was equal to or greater than the allogeneic MLR in normal subjects. This suggested that T lymphocytes from patients with SLE respond normally to allogeneic but not autologous B lymphocytes. B lymphocytes from patients with SLE did not stimulate allogeneic T lymphocytes normally (data not shown). The impaired autologous MLR in patients with SLE could have resulted from a failure of T cells to respond or B lymphocytes to stimulate an autologous MLR. This question was resolved by the study of three patients with SLE and their MLR identical healthy siblings. All three sets of these MLR identical siblings were also identical at the HLA-A and HLA-B loci.

Cellular basis of the impaired autologous MLR in patients with SLE. To distinguish between a failure of T lymphocytes to respond or a failure of B lymphocytes to stimulate an autologous MLR in patients with SLE, studies were carried out with SLE patients and their healthy MLR-identical siblings. T lymphocytes from a patient, her MLR-identical sibling, or an unrelated healthy subject were cultured together with B lymphocytes from each of these three subjects. The results of such an experimental protocol, obtained from three pairs of siblings, are presented in Table II. T cells from the patients with SLE responded normally when cultured with B cells from their healthy MLR-identical siblings or B cells from unrelated persons. In contrast, B cells from patients with SLE did not stimulate autologous or allogeneic T lymphocytes normally.

Three sets of siblings were studied. The MLR identity of the siblings is indicated by the fact that

B lymphocytes from the healthy sibling stimulated T cells from the patient and her sibling equally. The equivalent responses of T lymphocytes from either sibling to B lymphocytes from an unrelated individual also support this interpretation. Thus, the T-cell response of patients with SLE was comparable to that of healthy siblings. For this reason, the impaired autologous MLR in patients with SLE cannot be attributed to abnormal T-cell function.

In contrast, B cells from patients with SLE stimulated less thymidine incorporation by T lymphocytes from the patient, their siblings, or an unrelated subject than did B cells from their normal siblings. B lymphocytes from the patients with SLE stimulated only 25% as much thymidine incorporation by autologous or MLR identical T lymphocytes as did B lymphocytes from the normal sibling. Similarly, B lymphocytes from patients with SLE did not stimulate allogeneic T lymphocytes as well as did B lymphocytes from their siblings. Preincubation of the B lymphocytes from patients with SLE for 16 h at 37°C did not increase their stimulatory capacity (data not shown). This suggested that surface bound antibody or immune complexes do not inhibit the capacity of B lymphocytes from patients with SLE to stimulate autologous T cells.

DISCUSSION

We have presented evidence that B lymphocytes from patients with SLE do not normally stimulate the pro-

TABLE II
*Cellular Basis of the Impaired Autologous MLR in SLE**

	Stimulator B-cell donor		
	SLE patient	MLR identical normal sibling	Unrelated subject
	<i>Thymidine incorporated/culture</i> × 10 ⁻³		
SLE patient I	2.5	13.4	42.2
Normal sibling I	4.9	10.0	36.3
Unrelated subject X	21.1	38.6	11.8
SLE patient II	2.4	23.9	55.0
Normal sibling II	5.3	25.8	60.0
Unrelated subject Y	34.3	52.3	29.5
SLE patient III	2.3	9.8	22.4
Normal sibling III	1.6	9.3	20.0
Unrelated subject Z	25.0	39.6	3.0

* 10⁵ T lymphocytes were incubated with 2 × 10⁵-irradiated autologous or allogeneic B lymphocytes in 0.2 ml RPMI 1640 with 20% human serum for 6 d. Tritiated thymidine incorporation was measured during the last 24 h of incubation. Counts per minute given are the mean of triplicate cultures.

liferation of T lymphocytes. T lymphocytes from these patients, on the other hand, respond normally to B lymphocytes. These observations demonstrate that the impaired autologous MLR in SLE patients resulted from a defect in the stimulatory capacity of their B lymphocytes.

The impaired stimulatory capacity of B lymphocytes from patients with SLE did not appear to result from an increased number of monocytes. Although mononuclear cell preparations from patients with SLE have increased numbers of monocytes which may decrease mitogen-induced T-cell proliferation (8), in our studies lymphocyte preparations were depleted of monocytes. Antilymphocyte antibodies or immune complexes known to occur in serum from patients with SLE might inhibit the stimulatory capacity of B lymphocytes. This did not appear likely, as preincubation of the B lymphocytes under conditions that usually clear the lymphocyte surface of these ligands did not increase the stimulatory capacity of these cells. The most likely explanation for the impaired autologous MLR in patients with SLE appears to be either a loss of the B-lymphocyte subpopulation or an alteration in the surface membrane properties of that B-lymphocyte subpopulation which stimulates autologous T-lymphocytes.

Interaction of T and B lymphocytes may be necessary for the generation of suppressor activity. Preliminary studies, in our laboratory, have shown that during an autologous MLR, suppressor activity is generated. After 6 d of incubation, T lymphocytes incubated with autologous irradiated B lymphocytes inhibit polyclonal activation of autologous plaque-forming cells (unpublished observation). Patients with SLE have been reported to have less spontaneous and inducible suppressor activity (9, 10). The failure of patients with SLE to express suppressor activity may be a consequence of their impaired autologous MLR.

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