Failure of autologous mixed lymphocyte reactions between T and non-T cells in patients with systemic lupus erythematosus

(allogeneic mixed lymphocyte reactions/autoimmune diseases/suppressor activity/non-T cell subsets/T cell subsets)

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ABSTRACT   Normal human T cells proliferate vigorously when stimulated with autologous non-T cells. This autologous mixed lymphocyte reaction (MLR) between T and non-T cells was defective in patients with active systemic lupus erythematosus (SLE). In contrast, T cells and non-T cells from active SLE patients behaved normally as responding and stimulating cells, respectively, in the allogeneic MLR. The etiology of the impaired autologous MLR was further examined by studying the functional capacity of subsets of stimulating or responding cells. B cells, L cells, and monocytes from active SLE patients failed to stimulate autologous T cells but these cells effectively stimulated allogeneic T cells. Fc(IgG)⁺ T cells from active patients were unable to respond in both the autologous and allogeneic MLR; their Fc(IgG)⁻ T cells responded well in the allogeneic but not in the autologous MLR. The Fc(IgG)⁺ T cells, but not the Fc(IgG)⁻ T cells, from inactive SLE patients also failed to respond in the both autologous and allogeneic MLR. These studies indicate that patients with SLE have functionally defective Fc(IgG)⁺ T cells and a defective autologous MLR, both of which may contribute to impaired regulation of immune functions.

Systemic lupus erythematosus (SLE) is a complex multisystem autoimmune disease. One of its most prominent features is the presence of a wide variety of autoantibodies in the serum. In addition, several T cell defects have been described in patients with active SLE. It has been postulated that the excessive production of autoantibodies is due to some abnormality in B cell regulation by T cells (1, 2). More specifically, evidence has been presented to suggest a relative lack of suppressor T cell function in such patients (3–5).

In the course of our studies of suppressor T cell function in patients with SLE (5), we made the unexpected observation that, when lymphocytes from patients with SLE were used, the autologous mixed lymphocyte reaction (MLR) failed to occur. That is, whereas T cells from normal individuals proliferate vigorously in response to autologous non-T cells, T cells from SLE patients failed to proliferate normally in response to autologous non-T cells. In these studies we have investigated the reason for this defect in patients with SLE.

Because it has been recently proposed that "autologous MLR reflect a mechanism by which T lymphocytes regulate lymphocyte function" (6), the absence of such a reaction in the lymphocytes of patients with SLE is particularly provocative.

MATERIALS AND METHODS

Patients. All of the 33 patients studied satisfied the preliminary criteria of the American Rheumatism Association for the diagnosis of SLE. Their mean age was 30 years, and all but four patients were female. Patients receiving immunosuppressive drugs or high doses of corticosteroids (>10 mg of prednisone per day) were excluded. Activity of the SLE was based upon clinical disease as assessed blindly by two observers and was graded as active or inactive. Patients were considered active if they had clear-cut clinically identifiable objective signs and symptoms such as rash, pericarditis, and arthritis. If patients had only mild subjective symptoms they were included in the inactive group. The control group consisted of 38 healthy adults (12 females and 26 males) with a mean age of 32 years.

Purification of T Cells, Non-T Cells, and Monocytes. Blood was obtained early in the morning. The T and non-T cells were isolated from peripheral blood mononuclear cells as described (7). Briefly, the mononuclear cells were incubated with sheep erythrocytes (SRBC), and rosetted T cells were separated from non-T cells on Ficoll/Hypaque gradients. The non-T cells were depleted of monocytes by removal of cells adhering to petri dishes. Monocytes were obtained by collecting the cells adhering firmly to the dishes. Preparations of T cells were >95% pure as judged by rerosetting, and preparations of non-T cells consisted of 99% nonrosetting cells. More than 95% of "monocyte" preparations were monocytes as judged by morphology after Giemsa staining.

Fractionation of T Cells. Purified T cell preparations were subjected to further fractionation into Fc(IgG) receptor-positive T cells (Fc⁺ T cells) and negative T cells (Fc⁻ T cells) by using the preferential ability of Fc⁺ T cells to form rosettes with ox erythrocytes sensitized with rabbit IgG antibody to the erythrocytes (8). In these studies we only used IgG-coated ox erythrocytes and therefore only detected T cells with Fc receptors for IgG. Rosettes were first allowed to form by mixing such erythrocytes with T cells, and Fc⁺ T cells were then separated from Fc⁻ T cells on Ficol/Hypaque gradients. The Fc⁺ T cell preparations contained 80–85% rosetted cells with IgG-coated ox erythrocytes, whereas in the Fc⁻ T cell preparations the proportion of cells containing rosettes was less than 1%.

Fractionation of Non-T Cells. Previous studies in our laboratory have shown that non-T cells bearing surface membrane immunoglobulin (SmIg) did not form Fc-rosettes with sheep erythrocytes sensitized with the IgG fraction of rabbit anti-sheep erythrocyte serum, whereas non-T cells which did form Fc rosettes did not have SmIg (7). Therefore, each of these populations could be separated by the formation and density centrifugation of the rosettes. The rosetted and unrosetted non-T cells were designated L and B cells, respectively (7, 9). L cells, as defined here, have dense Fc receptors but not SmIg; B cells have SmIg but not dense Fc receptors detected by IgG-coated sheep erythrocytes. Both fractions were further

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Abbreviations: Fc⁺ T cells, T cells bearing Fc(IgG) receptors; Fc⁻ T cells, T cells not bearing Fc(IgG) receptors; MLR, mixed lymphocyte reactions; SLE, systemic lupus erythematosus; SmIg, surface membrane immunoglobulin.

depleted of monocytes by passage over rayon wool columns. The L cell fraction contained 85–95% Fc-rosetted cells, whereas in the B cell fraction less than 7% of the cells formed Fc rosettes.

Autologous and Allogeneic Mixed Lymphocyte Cultures. The separate cell populations were incubated overnight in RPMI 1640 (Grand Island Biological Co.) with 10% heat-inactivated fetal bovine serum (Microbiological Associates) at 37° in a 5% CO_2/95% air humidified environment and were used the following day.

Mixed lymphocyte cultures were established in triplicate and consisted of 0.2 ml of culture medium containing 1×10^5 responding T cells and 1×10^5 stimulating cells treated with mitomycin C (Sigma Chemical Co.) (7). The study of fractionated subpopulations as either stimulating or responding cells also used 1×10^5 cells of each subtype. All cultures were incubated for 144 hr at 37° in a 5% CO_2/95% air humidified environment. At 20 hr before the termination of the incubation period, 1 μCi of [*methyl*-^{3}H]thymidine ([^{3}H]dThd; 5 Ci/mmol; Amersham/Searle Corp.) was added to each culture well. At the end of the incubation period, the cells were processed on a microharvester and incorporation of [^{3}H]dThd was measured by liquid scintillation counting.

All data are expressed as the difference (Δ cpm) between the cpm from cultures containing stimulating cells (either autologous or allogeneic to responding T cells) and the cpm from cultures containing responding T cells alone.

RESULTS

Autologous MLR with Normal and SLE Lymphocytes. The lymphocytes of 38 normal individuals were studied in the autologous MLR. Purified T cells were used as responding cells and were stimulated to proliferate by autologous non-T cells. All normals responded vigorously (mean response, 9500 cpm; range 5100–27,500 cpm) (Fig. 1). No significant difference in the response was noted between males and females (data not shown). The 33 patients with SLE were divided on the basis of clinical disease activity into an "active" SLE group and an "inactive" disease group. Fifteen patients with inactive SLE responded with a mean Δ cpm of 5000 (range, 1600–11,400 cpm). This mean response was significantly different from that of the normal individuals ($P < 0.001$); however, in 9 of these 15 patients the incorporation of thymidine overlapped that observed in normal individuals. A group of 18 patients with active SLE had a mean Δ cpm of 940 for the autologous MLR (range, 400–2200 cpm). This mean value was significantly ($P < 0.001$) different from that of both normal individuals and patients with inactive SLE. Furthermore, there was no overlap between the active SLE group and the normal individuals.

The results presented here indicate that the patients with SLE had a defect in autologous MLR. Moreover, the lowest responses in the autologous MLR were observed in patients with active disease. The autologous MLR defect in SLE patients was most likely not due to steroid treatment (10), for the following reasons. First, despite the fact that 10 inactive patients had been treated with low doses of corticosteroids, 7 of them showed normal autologous MLR; second, in 4 active patients who had not received any medications, their autologous MLR was profoundly impaired. Third, in two active patients reinvestigated after corticosteroid-induced remission, the autologous MLR went from abnormal (400 and 1300 Δ cpm) to normal (5900 and 7000 Δ cpm). Taken together, these data provide strong evidence that the failure of autologous MLR in SLE patients is attributable to the disease itself.

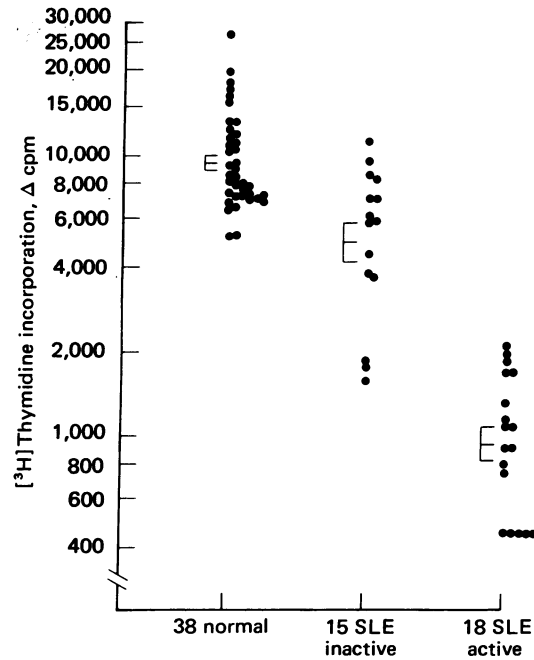In a dose-response experiment performed to determine

FIG. 1. Autologous MLR between T and non-T cells from normals, inactive SLE patients, and active SLE patients. Each circle represents an individual autologous MLR. The lines show geometric mean ± 1 SEM.

whether increasing the number of stimulating non-T cells could correct the defect in autologous MLR, 1×10^5 T cells were cultured with increasing numbers of autologous non-T cells. Whereas as few as 0.2×10^5 normal non-T cells induced proliferation of autologous T cells, 2×10^5 SLE non-T cells failed to induce proliferation of autologous T cells.

Allogeneic MLR with Normal and SLE Lymphocytes. T cells from both active and inactive patients functioned as well as normal T cells in the role of responding cells in the allogeneic MLR (Fig. 2). This was true regardless of the source of the stimulating non-T cells used. That is, non-T cells from normals and those from patients with either inactive or active SLE served adequately as stimulating cells. Thus, when unfrac-
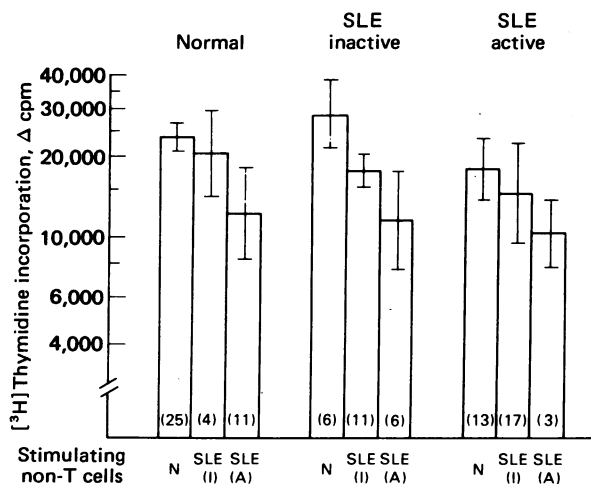

FIG. 2. Allogeneic MLR between T and non-T cells from normals, inactive SLE patients, and active SLE patients. The geometric mean responses and the range encompassed by ± 1 SEM are shown. The number tested is in parenthesis in each bar. Stimulating non-T cells: N, from normals; SLE(I), from inactive SLE patients; and SLE(A), from active SLE patients.

Table 1.   Stimulating capacities of different cell populations in autologous and allogeneic MLR*

| Donor of stimulating cells | Autologous MLR†, Δ cpm × 10⁻³ | | | | | Allogeneic MLR‡, Δ cpm × 10⁻³ | | | | |
	T cells	Unfrac-tionated non-T cells	B cells	L cells	Mono-cytes	T cells	Unfrac-tionated non-T cells	B cells	L cells	Mono-cytes
1. Normal	0.7	17.0	20.1	0.2	9.9	4.5	32.6	52.3	33.6	14.4
2. Normal	0.4	11.3	13.6	0.2	4.8	2.9	25.8	43.8	10.2	13.4
3. Normal	0.4	8.6	18.3	0.1	4.9	2.4	35.2	72.6	32.5	18.5
4. Normal	0.2	7.5	10.6	2.1	7.1	1.4	18.2	33.6	16.7	14.2
5. Inactive SLE	0.5	6.1	16.4	0.1	9.2	4.1	30.3	ND	ND	19.1
6. Inactive SLE	0.1	5.8	6.5	1.5	3.5	3.6	21.0	30.9	8.5	9.8
7. Active SLE	0.5	0.8	0.7	0.4	2.1	1.6	77.3	99.4	75.8	41.8
8. Active SLE	0.1	1.9	2.5	2.0	2.5	1.6	43.5	54.9	13.1	36.9

*The same stimulating cell subpopulations were used for the autologous MLR and the allogeneic MLR.
†Responding T cells were unfractionated and were from the same individual providing the stimulating cells.
‡Responding T cells were unfractionated and were from individuals allogeneic to those providing the stimulating cells. ND, not done.

tionated responder T cells are used, patients with SLE appear to have intact allogeneic MLR function.

Stimulating Capacities of Non-T Cell Subsets in Autologous and Allogeneic MLR. The explanation for the abnormal autologous MLR in patients with active SLE could be either a dysfunction of the participating cells (responding or stimulating cells) or inadequate numbers of participating cells. We first examined the percentages of B cells, L cells, and monocytes in the non-T cell population obtained from normal individuals and patients with inactive and active SLE. All three groups had similar proportions of these cells (data not shown).

We next investigated the stimulatory ability of subsets of non-T cells in the autologous and allogeneic MLR. In all these experiments, the same stimulating cells were used for both the autologous and the allogeneic MLR. In each of four normal instances, B cells were the most effective stimulating cells in both autologous and allogeneic MLR (Table 1). L cells failed to stimulate the autologous MLR; however, these cells were able to stimulate the allogeneic MLR. Monocytes could also act as stimulating cells in autologous and allogeneic MLR but were not as effective as B cells. Finally, in no instance were T cells able to stimulate the autologous or allogeneic MLR.

The purified B cells of two inactive SLE patients, like those of normal individuals, were the most effective stimulating cells in the autologous MLR; L cells were unable to stimulate the autologous MLR. Monocyte populations were also able to stimulate the autologous MLR. These non-T cell subsets from the inactive SLE patients were also effective stimulating cells in the allogeneic MLR. In two patients with active SLE, neither whole unseparated non-T cells nor fractionated non-T cell subsets stimulated autologous T cells. Nevertheless, these purified B cells, L cells, and monocytes from the same active SLE patients stimulated the allogeneic MLR as effectively as did those obtained from normal individuals. Finally, the fact that, with the active SLE patients, B cells separated from other non-T cells failed to stimulate autologous T cells makes unlikely the possibility that suppression by a non-B cell in the non-T cell population was the mechanism of the impaired autologous MLR in the active SLE patients.

Responding Capacities of T Cell Subsets in Autologous and Allogeneic MLR. To examine the role of responding SLE T cells in the abnormal autologous MLR, we investigated responsiveness of fractionated T cells (Fc⁺ T and Fc⁻ T cells) in autologous and allogeneic MLR. In these experiments the same absolute number of these fractionated T cells from normals and

inactive SLE and active SLE patients were used in the cultures.

With normal individuals, Fc⁺ T cells as well as Fc⁻ T cells responded well in both autologous and allogeneic MLR (Figs. 3 and 4). Selected patients with inactive SLE whose unfractionated T cells responded to a normal or almost normal degree in autologous MLR were then tested for the responding capacity of their fractionated T cells. The Fc⁻ T cell fractions obtained from these patients responded well in both autologous and allogeneic MLR. In contrast, only minimal responses were obtained with Fc⁺ T cell fractions from the inactive SLE patients either in autologous MLR or allogeneic MLR. When active SLE patients were studied, the Fc⁺ T cell fractions also responded abnormally in autologous MLR and allogeneic MLR. In addition, the Fc⁻ T cells from the active SLE patients were not stimulated in the autologous MLR.

In summary, responding lymphocytes from SLE patients were defective, and this defect appeared to be most prominent when Fc⁺ T cells were used.

Relationship between Concanavalin A-Induced Suppressor T Cell Activity and Autologous MLR. Concanavalin A-induced suppressor T cell activity of patients with SLE were studied by a previously described procedure (5, 11). Six patients with active SLE were studied simultaneously for suppressor T cell activity and autologous MLR. In every case, both suppressor cell generation and autologous MLR were markedly defective (Fig. 5). Lymphocytes from six normal individuals studied at the same time demonstrated normal activity for both functions.

DISCUSSION

During the past few years, several investigators have observed that purified human T cells can proliferate in response to autologous non-T cells (6, 10, 12). Although the nature of this reaction, called the autologous MLR, is uncertain, it has been proposed to be a mechanism by which T cells regulate B lymphocyte function (6, 13). In the present study we demonstrate that the autologous MLR is markedly impaired in patients with SLE, a disease in which there is a defect in immune control mechanisms. Furthermore, the defect in the autologous MLR was most profound in patients with active SLE and was less apparent in patients with inactive SLE. Kuntz (13) has also reported a defect in autologous MLR in patients with SLE.

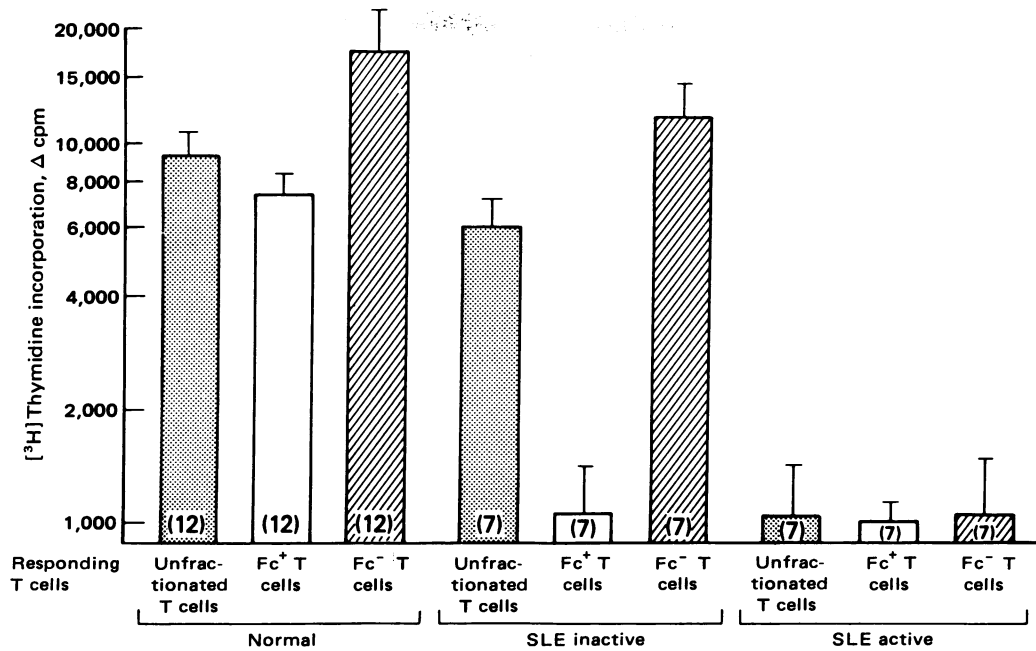There is universal agreement that the responding cell in the

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FIG. 3.   Responding capacities of unfractionated T cells, Fc$^+$ T cells, and Fc$^-$ T cells in the autologous MLR. The geometric mean responses + 1 SEM are shown. The number tested is in parentheses in each bar. Response of Fc$^+$ T cells was significantly lower in inactive SLE ($P < 0.001$) and in active SLE ($P < 0.005$) compared to normals. Response of Fc$^-$ T cells was significantly lower in active SLE ($P < 0.001$) compared to normals.

autologous MLR in normal individuals is a T cell (6, 10). However, there is a divergence of opinion concerning the precise nature of the stimulating cells. Although most investigators agree that, in general, a non-T cell is responsible, some investigators have ascribed most of the activity to L or K cells (6), whereas others have claimed that SmIg-bearing B cells are the best stimulating cells (14). Our own studies indicate that both B cells and monocytes, but not L cells, are the stimulating cells in autologous MLR. A closely related phenomenon is the autostimulation produced by long-term B cell lines (15, 16).

More recently it has been shown by Weksler and Kozak (17)

that the autologous MLR has the major attributes of an immunological reaction—that is, memory and specificity. Moreover, although the autologous MLR does not lead to the development of cytotoxic T cells directed against autologous non-T cells, it does lead to the generation of cytotoxicity directed toward antigens on third-party cells present during the autologous MLR (18). Phenomena quite similar to human autologous MLR also have been observed in several murine systems (19, 20). The animal studies support the hypothesis that autologous MLR may be an important correlate of cell interaction in normal immune responses (19, 20).
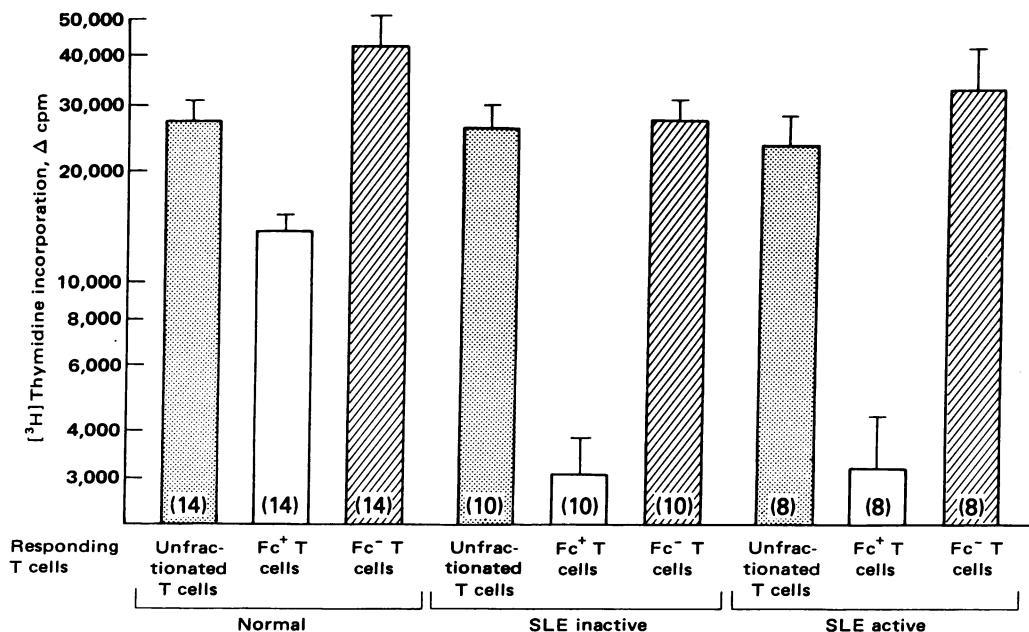

FIG. 4.   Responding capacities of unfractionated T cells, Fc$^+$ T cells, and Fc$^-$ T cells in the allogeneic MLR. The geometric mean responses + 1 SEM are shown. The number tested is in parentheses in each bar. The response of Fc$^+$ T cells was significantly ($P < 0.001$) lower in inactive SLE and in active SLE compared to normals.
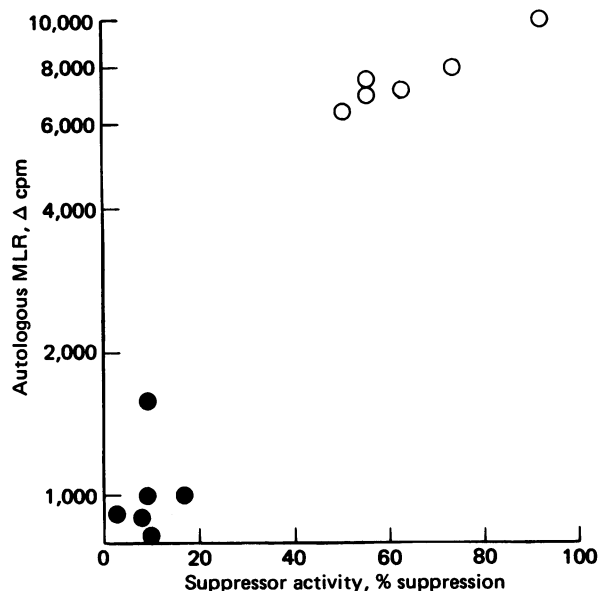
FIG. 5.   Relationship between concanavalin A-induced suppressor
T cell activity and autologous MLR with normal (O) and SLE (●)
cells. Suppressor activity was evaluated from the ability of concana-
valin A-activated T cells to suppress allogeneic responses by T cells
autologous with concanavalin A-activated T cells (5, 11).

To explore further the defect in autologous MLR in patients
with SLE, a number of additional studies were performed. We
first examined the role of the stimulating non-T cells. Increasing
the number of stimulating non-T cells did not correct the defect.
The same SLE non-T cells that failed to stimulate in the au-
tologous MLR were effective stimulating cells of an allogeneic
MLR. However, this observation does not entirely eliminate
the possibility that the defect is in the stimulating non-T cells,
because the structures on these cells responsible for the allo-
geneic MLR may be separate from those capable of stimulating
autologous MLR.

Another possibility is that the defect in autologous MLR re-
sides in the responding T cell population. To explore this pos-
sibility we examined various T cell subpopulations for their
ability to act as responding cells in autologous and allogeneic
MLR. The overall results of these studies indicate a defect in
the Fc⁺ T cell subpopulation in patients with SLE. Of interest
in this regard is the recent observation by Moretta *et al.* (8) that
Fc⁺ T cells are the cells that act as suppressor cells. In the
present study, we observed that SLE patients who were de-
fective with regard to autologous MLR were also defective with
regard to concanavalin A-induced suppressor T cell generation.

The defects of both autologous MLR and suppressor function
observed in patients with SLE appear to be intimately related
and may represent the same event.

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