Decreased Production of and Response to Interleukin-2 by Cultured Lymphocytes from Patients with Systemic Lupus Erythematosus

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Abstract. We studied the production of and response to interleukin-2 (IL-2) by peripheral blood T lymphocytes from 19 systemic lupus erythematosus (SLE) patients who received no treatment at the time they were studied. Eight had active disease and the rest were in remission. Results were compared with those obtained in 12 healthy subjects of similar age. T cells from SLE patients, whether activated with phytohemagglutinin or in autologous mixed lymphocyte reactions, were found to yield little IL-2, to have a low response to IL-2 from its own, or other sources, and to absorb IL-2 poorly. IL-2 produced by SLE cells, albeit scant, was absorbed normally by activated T cells from normal subjects.

Our findings may contribute to the understanding of the immunoregulatory defect in SLE.

Introduction

The T cell growth factor interleukin-2 (IL-2) is an antigen-nonspecific, soluble factor produced by T lymphocytes upon activation (1, 2). In turn, activated T lymphocytes are themselves the responding cells to IL-2 and become responsive to it after they enter the G1 phase of the cell cycle (3). Recent studies indicate that IL-2 is important in supporting the immunoregulatory functions of T lymphocytes (4).

Systemic lupus erythematosus (SLE) is a complex autoimmune disease that may result from defective functions of the immunoregulatory T cell circuits (5). The production of and response to IL-2 have not been previously studied in cells from SLE patients, but in recent communications both have been found diminished in NZB/NZW F1 and MRL/1pr mice, animal models of SLE (6, 7).

We report here evidence of a decreased production of and response to IL-2 by activated T lymphocytes from patients with SLE as compared with those from normal subjects. This decrease was more marked in patients with active than in those with inactive disease. Our findings may contribute to the characterization of the immunoregulatory aberration that causes and/or perpetuates SLE.

Methods

Subjects. We studied 19 SLE patients who fulfilled at least four of the preliminary criteria for classification of this disease (8). All but one were females. Their age ranged from 25 to 58 yr (mean, 30.2). No patient was receiving corticosteroids, antimalarial, or immunosuppressive drugs nor had received them for at least 3 mo. Nonsteroidal anti-inflammatory agents were stopped at least a week before the time of the study in a few patients who were taking them.

Patients fell into two distinct categories in regard to disease activity. Eight had active disease requiring treatment and were studied before its initiation. The other 11 have been in remission from 1 to 8 yr and have had all medication withdrawn. When their disease was active they all had amply fulfilled the aforementioned classification criteria.

We studied as controls 12 healthy volunteers whose ages ranged from 23 to 35 yr with a mean of 28.1. Six were female.

Cells' separation and purification. Peripheral venous blood drawn into heparinized syringes (10 IU/ml) was diluted in an equal volume of phosphate-buffered saline pH 7.4 and centrifuged on Ficoll-Hypaque cushions of a 1.077 g/ml density.

Mononuclear cells (MNC) were recovered at the interface and washed twice in Minimum Essential Medium (MEM). T and non-T cells were purified from MNC by rosetting with sheep erythrocytes. For this, we mixed 107 MNC in 1 ml of MEM with 2 ml of a 2% suspension of sheep erythrocytes and 0.5 ml of gamma globulin-free fetal calf serum (FCS) (Gibco Laboratories Grand Island Biologicals Co., Grand Island, NY). The mixture was centrifuged 10 min at 130 g and incubated 1 h at 4°C. While still in the cold the pellets were resuspended gently and placed on Ficoll-Hypaque for separation of T and non-T cells from the pellet and interface.
respectively. The erythrocytes were lysed with distilled water and each population of cells was washed two times in MEM and resuspended in complete medium (RPMI 1640, Gibco Laboratories) with the addition of penicillin (100 U/ml), streptomycin (100 μg/ml), and L-glutamine 2 mM (Sigma Chemical Co., St Louis, MO). T cells contained >93% cells capable of rosetting with sheep erythrocytes and non-T cells had <5% of such cells.

Production of IL-2. We stimulated T cells to produce IL-2 both with phytohemagglutinin (PHA) and in autologous mixed lymphocyte reactions (AMLR). PHA stimulation was done essentially as described by Bonnard et al. (9). Briefly, after 24 h of culture in RPMI with 5% FCS and 1% PHA, in a 5% CO₂, 100% humidity, 37°C atmosphere, MNC were washed three times in RPMI, resuspended in it, and incubated similarly for another 24 h without PHA or FCS in 17 X 100-mm round bottom plastic tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA). The tubes were then centrifuged at 2,000 rpm for 15 min to collect the supernatants. These were filtered through 0.45 μm filters (Millipore Corp., Bedford, MA) and stored at -20°C.

Stimulation in AMLR was done by co-culturing in plastic tubes 3 X 10⁶ T cells with 3 X 10⁶ mitomycin-treated autologous non-T cells in RPMI with 5% FCS for 48 h. The supernatants were collected, filtered, and stored as above.

Assay for IL-2 activity. Normal human T cells activated with PHA were held in culture for at least 2 wk in RPMI supplemented every 4 d after the 1st wk in culture with both FCS and lectin-free IL-2 (Associated Biomedic Systems, Buffalo, NY). The cells were used 4 d after the last addition of IL-2 or the assays of IL-2 activity. They were washed in MEM and placed in microculture plates (10⁴ T cells) in 100 μl of RPMI with 5% FCS. The supernatants being tested were added at four different dilutions. The plates were incubated at 37°C for 48 h. Controls with resting cells were included and all assays were done in triplicate. After 36 h in culture we added 0.5 μCi of \[^{3}H\]thyidine with a specific activity of 77.2 Ci/mmol. (New England Nuclear, Boston, MA). At the end of the culture we harvested the cells automatically into glass fiber filter strips, placed them in a standard scintillation mixture for counting in a Packard tri-carb spectrometer (Packard Instruments Co., Inc., Downers Grove, IL). Cellular proliferation induced by the supernatants was expressed as the mean counts per minute of triplicate cultures.

Response of cells to IL-2. To test the response of T cells from patients or controls to IL-2, we activated them in 7-d AMMLR, or with PHA during 4 d. After this, we separated the T cells from the non-T cells by rosetting with sheep erythrocytes and Ficoll-Hypaque gradients. The microculture conditions, the addition of normal or SLE cells' supernatants as a source of IL-2 activity, or of reference lectin-free IL-2 (Associated Biomedic Systems), the time and dose of \[^{3}H\]thyidine added, and the harvesting and counting procedures were as detailed above.

Absorption of IL-2 activity. To determine the IL-2 nature of the factor capable of activating T cells present in the supernatants these were absorbed with cells that had been maintained for >15 d in culture with IL-2, but had been deprived of it 4 d before absorption. We also tested the capacity of patients' or controls' cells activated in a 7-d AMLR to bind lectin-free IL-2 (Associated Biomedic Systems). The respective assays were done with (6 X 10⁶ T cells incubated with 1 ml of supernatant or 1 ml (4 U) of commercial IL-2 during 4 h at 37°C.

Absorption of IL-2 activity was determined by the decrease of the \[^{3}H\]thyidine incorporation elicited on T cells by the absorbed supernatants or commercial IL-2, compared with the base line of the \[^{3}H\]thyidine incorporation elicited by the unabsorbed ones and expressed as their percentage.

RESULTS

Production of IL-2 activity by lymphocytes from patients or controls. As shown in Fig. 1, T cells from SLE patients produced significantly less IL-2 activity in comparison with those from normal subjects (P < 0.0001 at all four dilutions of supernatant), as reflected by the \[^{3}H\]thyidine incorporation of normal activated T cells exposed to their supernatants. Findings were similar to those shown in Fig. 1 when PHA was left throughout the 48 h of culture rather than removed after 24 h (at 1:2 dilution, normals [n = 3]: 9,216 ± 72, active SLE [n = 2]: 2,804 ± 396, inactive SLE [n = 4]: 4,101 ± 70).

T cells from SLE patients with active disease produced significantly less IL-2 than those from patients with inactive disease (P < 0.005 at all four dilutions). Decreased production of IL-2 by T cells from SLE patients occurred with cells activated with PHA as well as with cells activated in AMLR (Fig. 1, a and b).

All SLE patients with active disease and all but one with inactive disease had PHA-stimulated T cells that produced less IL-2 activity than the mean −2 SD of that produced by cells from normal subjects. Only one patient with inactive disease had a production of IL-2 activity by her T cells upon stimulation in AMLR similar to that of normal but she was not the same patient who had a normal IL-2 production upon PHA activation. Therefore, no patient's cells yielded normal levels of IL-2 activity with both stimuli.

To rule out the possibility that an antibody produced by SLE cells in culture might be present in their supernatants and could therefore interfere with the IL-2 activity, the supernatants of PHA-stimulated cells from three SLE patients were partially purified on affinity Sepharose 4B columns with anti-IgG or anti-IgM antibody to remove these immunoglobulins. Filtrates were dialyzed against phosphate-buffered saline and concentrated to their original volume. Analysis by nephelometry and immunodiffusion showed them to be devoid of IgG or IgM, respectively. Response of normal activated T cells to these fractions was similar to that obtained with the crude supernatant (1:2 dilution: crude 3,879 ± 271, IgG-free 3,678 ± 139, IgM-free 4,002 ± 117, cpm). Mixture of known amounts of commercial IL-2 with SLE supernatant, caused a mean decrease in the proliferative response of normal stimulated cells of only 4% as compared with IL-2 similarly mixed with complete medium (three experiments).

Response to IL-2 by SLE or control T lymphocytes. The response of T lymphocytes from SLE patients to IL-2 was lower than that of T lymphocytes from normal subjects whether activated in AMLR or with PHA
cells to subjects. Responses to supernatants from T or inactive controls or absorb to cells those cyttes as 2 activity yielded IL-2 absorption from IL-2 patients, but IL-2 activity, which was less significant within the normal activated cells from patients with active and those of patients with inactive disease were significant at the P < 0.001 level except in the 1:20 dilution with PHA activation (P < 0.0005). The differences between cells from patients with active and those of patients with inactive disease were significant at the P < 0.001 level in all instances except in the 1:20 dilution with AMLR activation (P < 0.005), as determined by t tests.

**DISCUSSION**

We can postulate that the activity present in the supernatants from PHA or AMLR-activated T cells is IL-2 on the following grounds: (a) it is a product of T cells from patients with active or inactive disease. These differences were noticeable with all dilutions of normal IL-2. Response of T cells from SLE patients to their own IL-2 activity was decreased proportionately to their diminished response and to the lower amounts of this factor found in their supernatants (data not shown). None of the SLE patients, whether active or inactive, showed T cell responses to IL-2 activity within the mean ± SD of the normal subjects. Somewhat higher response to IL-2 by nonstimulated T cells from SLE patients than by those from normal controls was noticed (Table I).

**Absorption of IL-2 by T lymphocytes from SLE patients or controls.** Fig. 2 shows the percent absorption of IL-2 by T lymphocytes from normal controls and SLE patients with active or inactive disease. T cells from all SLE patients had decreased capacity to absorb IL-2 activity, but this was more pronounced in those from patients with active disease. Interleukin-2 activity yielded by T cells from SLE patients was as readily absorbed by normal activated T lymphocytes as that produced by T cells from normal subjects (percent absorption: 73.75±7.0 vs. 74.8±2.4, mean±SEM of five experiments, respectively).

**FIGURE 1** Proliferative response induced on T cells by four different dilutions of the supernatants of activated T cells from normal patients (○), patients with inactive SLE (Δ), and patients with active SLE (▪). The response to the supernatant of normal nonactivated T cells is also shown (◊). Fig. 1a shows the mean responses (±SEM) with supernatants from cells activated in AMLR and Fig. 1b those of cells activated with PHA. The differences between normal activated T cells and those from SLE patients, both active and inactive, were significant at the P < 0.0001 level except in the 1:20 dilution with PHA activation (P < 0.0005). The differences between cells from patients with active and those of patients with inactive disease were significant at the P < 0.001 level in all instances except in the 1:20 dilution with AMLR activation (P < 0.005), as determined by t tests.
TABLE I

$[^3]H$Thymidine Incorporation by T Cells from Normal Subjects or SLE Patients in Response to IL-2

<table>
<thead>
<tr>
<th>Cell's source</th>
<th>Dilution</th>
<th>1:2</th>
<th>1:4</th>
<th>1:10</th>
<th>1:20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal subjects</td>
<td>Stimulated in AMLR (6)*</td>
<td>8,950±215</td>
<td>7,563±691</td>
<td>5,496±718</td>
<td>4,394±183</td>
</tr>
<tr>
<td></td>
<td>With PHA (6)</td>
<td>8,846±271</td>
<td>7,831±208</td>
<td>6,130±121</td>
<td>5,084±89</td>
</tr>
<tr>
<td></td>
<td>Not stimulated (6)</td>
<td>1,206±48</td>
<td>846±131</td>
<td>636±151</td>
<td>418±39</td>
</tr>
<tr>
<td>SLE patients</td>
<td>Stimulated in AMLR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Active disease (8)</td>
<td>1,201±419‡</td>
<td>1,219±825</td>
<td>1,069±881</td>
<td>971±846</td>
</tr>
<tr>
<td></td>
<td>Inactive disease (10)</td>
<td>3,776±750§</td>
<td>3,395±1,104</td>
<td>3,081±919</td>
<td>2,250±748</td>
</tr>
<tr>
<td></td>
<td>Stimulated with PHA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Active disease (4)</td>
<td>1,627±151¶</td>
<td>1,380±145§</td>
<td>1,212±71¶</td>
<td>990±99§</td>
</tr>
<tr>
<td></td>
<td>Inactive disease (7)</td>
<td>4,285±270§</td>
<td>3,815±251§</td>
<td>3,257±195§</td>
<td>2,521±213§</td>
</tr>
<tr>
<td></td>
<td>Not stimulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Active disease (8)</td>
<td>1,585±318</td>
<td>1,486±210¶</td>
<td>976±38¶</td>
<td>647±79</td>
</tr>
<tr>
<td></td>
<td>Inactive disease (10)</td>
<td>1,459±976</td>
<td>1,376±818</td>
<td>466±305</td>
<td>396±64</td>
</tr>
</tbody>
</table>

* Parentheses indicate number of subjects in each group.
† Mean±SEM.
‡ t test, P < 0.0001 vs. normal stimulated cells.
§ t test, P < 0.01 vs. normal stimulated cells.
¶ t test, P < 0.001 vs. normal stimulated cells.

The finding of decreased production of and response to IL-2 by T cells from SLE patients is not surprising. IL-2 is known to support T cell proliferation under mitogenic (12) or antigenic stimuli (13) as well as their response in AMLR (14). It also contributes to the generation of natural cytotoxicity (4), to the abrogation of the suppressor cell function induced by concanavalin-A (15), and to the promotion of helper cell function (4, 16). It may contribute to the postthymic maturation of T cells (17). All of these functions have been found altered in SLE (5, 18–23). Furthermore, NZB/NZW F1 hybrids and MRL/lpr mice, animal models of SLE, have been recently shown to have a similar defect in the response to and production of IL-2 (6, 7).

The culture conditions in our assays rules out the possibility that serum factors prevented the interaction of IL-2 with surface receptors on cells from SLE patients. Indeed, unstimulated T cells from SLE patients seemed to respond the same or better to IL-2 activity than those of normal subjects.

The abnormalities of T cell function found in SLE have been ascribed to lymphocytotoxic (24) or antinuclear antibodies (25) causing deletion of T cell subpopulations. Specific decrease of Ty cells (26), autologous rosette forming T cells (5), and OKT 5–8 positive cells (27) have all been found in SLE patients, particularly in those with active disease. The decreased pro-

**Figure 2** Percent absorption of IL-2 activity by T cells from normal controls and patients with inactive or active SLE.
duction of and response to IL-2 by T cells from SLE patients could either be due to an intrinsic defect of the T cells in their capacity to become activated and/or to produce receptors to IL-2 or to loss of a T cell subpopulation, either primarily or due to autoantibodies. The presence of the defect in patients with long inactive disease might point to a primary defect of T cells and their maturation influenced by thymus related factors (5). This is, however, not conclusive and remains, as many other questions about SLE, currently unanswerable.

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REFERENCES