Impairment of CD8+ T Suppressor Cell Function in Patients with Active Systemic Lupus Erythematosus

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Impairment of CD8+ T Suppressor Cell Function in Patients with Active Systemic Lupus Erythematosus

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Alteration of T cell suppression function has been recognized in patients with systemic lupus erythematosus (SLE). Recently, CD8+ T suppressor lymphocytes (CD8+ Ts) have been generated in vitro by incubating purified CD8+ T cells with IL-2 and GM-CSF. Using this method, we generated CD8+ Ts from patients affected by SLE. No major differences were found in the CD8+ Ts phenotype between SLE patients and healthy subjects. CD8+ Ts from SLE patients with active disease did not inhibit the anti-CD3 mAb-induced proliferation of autologous PBMC, whereas CD8+ Ts from SLE patients in remission exerted an inhibitory activity comparable to normal subjects. The inhibitory effect of CD8+ Ts cells was neither mediated by cytotoxic activity nor by apoptosis induction. Two cytokines, IFN-γ and IL-6, were found to be responsible for the function of CD8+ Ts. In fact, counteraction of CD8+ Ts suppression activity was obtained by blocking IFN-γ with a specific Ab or by inhibiting CD8+ T-mediated IL-6 secretion by an antisense oligonucleotide. Interestingly, CD8+ Ts from SLE patients showed a peculiar cytokine pattern characterized by an impaired secretion of IL-6 and an increased secretion of IL-12. Thus, it appears that an altered balance between inhibitory (IL-6) and stimulatory (IL-12) cytokines might be responsible for the functional impairment of CD8+ Ts in SLE patients. The Journal of Immunology, 2001, 166: 6452–6457.

Autoimmune diseases are characterized by the reactivity of lymphocytes against endogenous Ags. Among the pathogenic mechanisms proposed to explain autoimmune phenomena, the down-regulation of T suppressor lymphocyte function has been postulated (1). Interestingly, either reduction or functional impairment of CD8+ T suppressor lymphocytes (CD8+ Ts) has been observed in relapse phases of different immunomediated diseases such as multiple sclerosis (2), Crohn’s disease (3), and myasthenia gravis (4). Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the systemic deposition of immunocomplexes (5). Several immunological abnormalities have been observed in SLE patients, including the alteration of T cell suppressor function (6). In particular, the incapacity to inhibit both B and T cell proliferation and Ab or autoantibody production have been reported (7). However, a detailed phenotypic and functional characterization of T suppressor lymphocytes in SLE is lacking.

Balashov et al. (2) recently reported the possibility to generate a subpopulation of CD8+ Ts in vitro by coincubating autologous monocytes and purified CD8+ T cells in the presence of IL-2 and GM-CSF. The authors also reported that this lymphocyte subpopulation was functionally impaired in patients affected by multiple sclerosis, a disease in which autoimmune mechanisms seem to play a major role.

In this study, we investigated the possibility that abnormalities in CD8+ Ts could be found in SLE patients. To this end, CD8+ Ts have been generated from SLE patients and healthy subjects and their function has been extensively analyzed.

Materials and Methods

Patients

Fifteen female patients, 25–48 years old, affected by SLE according to the American College of Rheumatology criteria for the diagnosis of SLE (8) were enrolled (Table I). Seven patients were affected by active SLE and eight patients were in remission as assessed by calculation of the SLEDAI index. All patients were under treatment with oral prednisone (5–10 mg/day). Three patients affected by acute SLE were studied before and after successful aggressive immunosuppressive treatment. Among them, one was treated with i.v. high-dose methylprednisolone (1 g/day for 3 consecutive days) and two received i.v. high-dose cyclophosphamide (1 g/mo for 6 mo). Nine healthy subjects (3 males and 6 females, 26–45 years old) were studied as controls.

Generation of CD8+ Ts

CD8+ Ts were generated as described by Balashov et al. (2). Briefly, PBMC were isolated by centrifugation on a Ficoll-Hypaque gradient for 30 min at 1800 rpm. PBMC were incubated in RPMI 1640 culture medium (Sigma, Milan, Italy) added with 10% FCS (Sigma), 2% glutamine (Sigma), and 0.1% penicillin-streptomycin (Sigma) in culture flasks (Corning Costar, Cambridge, MA) at 37°C overnight. After removal of nonadherent cells, monocytes were collected by gently scraping with a cell lifter (Corning Costar) and irradiated (2500 rad). CD8+ T lymphocytes were purified from nonadherent cells by magnetic beads separation. To positively select CD8+ T lymphocytes, magnetic beads coated with anti-CD8 mAb (Dyna beads M-450 CD8; Dynal, Great Neck, NY) and the CD8 Detach bead (Dynal) were used according to the manufacturer’s instructions. The positive selection procedure was repeated until the cell population was highly enriched in CD8+ T cells (>95%) as demonstrated by flow cytometric analysis. CD8+ T lymphocytes (1 × 10^6 cells) were incubated with autologous irradiated monocytes (2.5 × 10^6 cells) in 96-well flat-bottom plates (Corning Costar) in RPMI 1640 medium containing 20 U/ml IL-2 (Chiron, Emeryville, CA) and 10 ng/ml GM-CSF (Roche, Milan, Italy) at 37°C up to 7 days. At the end of the incubation, nonadherent cells were
collected and the CD8⁺ Ts population was further purified by positive selection with magnetic beads as described above. Each single CD8⁺ Ts cell preparation was used for one single experiment.

In the case of patients with SLE in remission, CD8⁺ Ts were generated after at least 3 mo of stable disease remission.

**Monoclonal Abs**

mAbs to CD2, CD3, CD4, CD8, CD56, CD28, CD11b, LFA-1, and CD45 Ags were purchased from Coulter Immunotech (Miami, FL); mAb UCTH-1 to CD3 (9) and mAb to p58.1, p58.2, p58.3, p70, and CD94 NK receptors (10) were a gift from Prof. A. Moretta (University of Genoa); and mAb W6/32 and mAb Q5/13 to HLA class I and class II Ags, respectively (11, 12), were a gift from Prof. S. Ferrone (Roswell Park Cancer Institute, Buffalo, NY).

**Phenotypic analysis of CD8⁺ Ts and characterization of cytokine production by intracellular staining**

The phenotypic analysis of CD8⁺ Ts was performed by indirect immunofluorescence. Briefly, 1 × 10⁶ cells were incubated in 100 μl of HBSS (Sigma) with the appropriate mAb at 4°C for 30 min. After extensive washings with HBSS, cells were incubated with FITC-labeled goat anti-mouse IgGs (Caltag, South San Francisco, CA) for 30 min at 4°C in the dark. After further washings, cells were analyzed by the Epics Profile II flow cytometer (Coulter Electronics, Hialeah, FL).

Intracellular staining of CD8⁺Ts was performed by first incubating the cells with a FITC-conjugated anti-CD8 mAb for 10 min in the dark at room temperature. After washings, cells were fixed and permeabilized by using the Fix & Perm cell permeabilization kit (Caltag) following the manufacturer’s instructions, washed, and incubated with a PE-conjugated anti-IFN-γ (Caltag), anti-IL-6 (BioErgonomics, St. Paul, MN), or anti-IL-12 mAb (Caltag) at 15 min at 4°C. Isootype-matched, FITC-conjugated, or PE-conjugated mAbs, specific for irrelevant Ags, were used as negative controls. Analysis of double-positive cells was performed by cytometer.

**PBMC proliferation in the presence of CD8⁺ Ts and cytokines**

Nonadherent PBMC were incubated with the anti-CD3 mAb UCHT-1 (5 μg/ml) at 4°C for 2 h, washed, counted with trypan blue dye, and seeded in a 96-well flat-bottom plate (1 × 10⁵ cells/well) in the presence of autologous irradiated monocytes (4 × 10⁵ cells/well). Autologous CD8⁺ Ts were added to wells at 8 × 10⁴, 4 × 10⁴, and 2 × 10⁴ cells/well concentrations. Control cultures were performed in the absence of CD8⁺ Ts. In some experiments, PBMC preincubated with the anti-CD3 mAb UCHT-1 and autologous irradiated monocytes were added to the bottom well while CD8⁺ Ts were added to the top well, respectively, of a Transwell system (Corning Costar). In another series of experiments, 10 μg/ml of the anti-IFN-γ mAb 69 (gift from Prof. A. Sinigaglia, Roche, Milan, Italy) was daily added to cultures. In additional experiments, CD8⁺ Ts and/or macrophages were preincubated with the oligonucleotide 5'-TCCTGGGGGTTACTG-3' (antisense of IL-6 genetic sequence; Genset, La Jolla, CA) or with the oligonucleotide 5’-CAGTACCCCCAGG-3’ (sense of IL-6 genetic sequence; Genset). To favor oligonucleotide penetration into cells, DOTAP Liposomal Transfection Reagent (Boehringer Mannheim, Indianapolis, IN) was used according to the manufacturer’s instructions. Cells were then added to anti-CD3 mAb-stimulated PBMC cultures as described above.

In a further series of experiments, PBMC preincubated with the anti-CD3 mAb UCHT-1 and autologous irradiated monocytes were cultured in the presence of either recombinant IL-6 (Genzyme, Cambridge, MA) at concentrations ranging from 100 to 2000 ng/ml or recombinant IL-12 (gift from Prof. L. Adorini, Roche) at 3.6 and 36 ng/ml concentration. All cultures were performed in triplicate for 5 days at 37°C. [³H]Thymidine (0.5 μCi) was added to each well 12 h before the end of the incubation. Cells were then collected by a cell harvester and the incorporated radioactivity was detected by a beta counter (Wallac, Turku, Finland).

**Cytotoxic assays**

Cytotoxic assays were performed to evaluate: 1) the cytotoxic activity against autologous PHA-activated PBMC; 2) the redirected cytotoxicity against mouse mastocytoma P815 cells preincubated with the anti-CD3 mAb UCHT-1; and 3) the natural cytotoxicity against human erythroleukemia K562 cells.

In the first assay, CD8⁺ Ts were incubated for 4 h at 37°C with ⁵¹Cr-labeled autologous PBMC activated with PHA (1:1000) for 72 h at the E:T ratio of 20:1. At the end of the incubation period, plates were centrifuged (1000 rpm for 5 min), 100 μl of supernatant was collected from each well, and the radioactivity was measured as cpm in a gamma counter (Wallac). The radioactivity detected in the supernatant of PHA-activated PBMC (5 × 10⁵ cells) incubated with Triton X-100 or cultured in RPMI 1640 medium

### Table I. Patient characteristics

<table>
<thead>
<tr>
<th>Patient Age (yr)</th>
<th>Main Clinical Manifestation</th>
<th>SLE Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Serositis</td>
<td>Active</td>
</tr>
<tr>
<td>2</td>
<td>Nephritis</td>
<td>Active</td>
</tr>
<tr>
<td>3</td>
<td>Nephritis</td>
<td>Active</td>
</tr>
<tr>
<td>4</td>
<td>Anti-phospholipid Ab syndrome</td>
<td>Active</td>
</tr>
<tr>
<td>5</td>
<td>Arthralgia/ myalgia</td>
<td>Active</td>
</tr>
<tr>
<td>6</td>
<td>Hepatitis</td>
<td>Active</td>
</tr>
<tr>
<td>7</td>
<td>Serositis</td>
<td>Active</td>
</tr>
<tr>
<td>8</td>
<td>Serositis</td>
<td>In remission</td>
</tr>
<tr>
<td>9</td>
<td>Arthralgia/myalgia</td>
<td>In remission</td>
</tr>
<tr>
<td>10</td>
<td>Arthralgia/myalgia</td>
<td>In remission</td>
</tr>
<tr>
<td>11</td>
<td>Neuropathy</td>
<td>In remission</td>
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<tr>
<td>12</td>
<td>Nephritis</td>
<td>In remission</td>
</tr>
<tr>
<td>13</td>
<td>Arthralgia/myalgia</td>
<td>In remission</td>
</tr>
<tr>
<td>14</td>
<td>Arthralgia/myalgia</td>
<td>In remission</td>
</tr>
<tr>
<td>15</td>
<td>Fever-pancytopenia</td>
<td>In remission</td>
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</table>

### Table II. Phenotypic analysis of CD8⁺ Ts cells

<table>
<thead>
<tr>
<th>Ag</th>
<th>CD2</th>
<th>CD3</th>
<th>CD4</th>
<th>CD56</th>
<th>P58.1</th>
<th>P58.2</th>
<th>P58.3</th>
<th>P70</th>
<th>CD94</th>
<th>CD28</th>
<th>CD11b</th>
<th>LFA-1</th>
<th>CD45</th>
<th>HLA class I</th>
<th>HLA class II</th>
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<tr>
<td>Healthy</td>
<td>99</td>
<td>99</td>
<td>0</td>
<td>98</td>
<td>12</td>
<td>3</td>
<td>6</td>
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<td>10</td>
<td>13</td>
<td>5</td>
<td>1</td>
<td>95</td>
<td>99</td>
<td>99</td>
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<tr>
<td>SLE in remission</td>
<td>99</td>
<td>99</td>
<td>0</td>
<td>98</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>56</td>
<td>99</td>
<td>99</td>
</tr>
<tr>
<td>Active SLE</td>
<td>99</td>
<td>99</td>
<td>0</td>
<td>97</td>
<td>11</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>72</td>
<td>99</td>
<td>99</td>
</tr>
</tbody>
</table>

Data are expressed as percent positive cells.
was considered as the maximum and minimum lysis, respectively. All cultures were performed in triplicate. The percentage of lysis was calculated by the following formula: percent lysis = (sample cpm – minimum cpm)/(maximum cpm – minimum cpm) × 100.

In the second assay, CD8⁺ Ts were incubated for 4 h at 37°C with ⁵¹Cr-labeled P815 cells preincubated with the anti-CD3 mAb UCHT-1 (5 μg/ml) at the E:T ratio of 20:1. In the third assay, CD8⁺ Ts were incubated for 4 h at 37°C with ⁵¹Cr-labeled K562 cells at the E:T ratio of 50:1. The assays were then performed as described above.

CD8⁺ Ts-induced PBMC apoptosis
CD8⁺ Ts (4 × 10⁵ cells) were added to the top well while autologous irradiated monocytes (4 × 10⁵ cells) and nonadherent PBMC (5 × 10⁵ cells) preincubated with the anti-CD3 mAb UCHT-1 (5 μg/ml) were seeded in the bottom well, respectively, of a Transwell system (Corning Costar). After 5 days, PBMC (5 × 10⁵ cells) were collected and incubated in 100 μl of HBSS containing propidium iodide (50 μg/ml), sodium citrate (0.1%), and Triton X-100 (0.1%) at 4°C in the dark for 12 h according to Nicoletti et al. (13). The percentage of apoptotic cells was evaluated by flow cytometric analysis.

Cytokine determination
CD8⁺ Ts were generated from each SLE patient and healthy subject included in the study. CD8⁺ Ts (1 × 10⁵ cells) and irradiated autologous monocytes (4 × 10⁵ cells) were cocultured in culture medium alone or in culture medium containing IL-2 (50 U/ml) or anti-CD3 mAb UCHT-1 (5 μg/ml) for 24 h at 37°C. At the end of the incubation period, supernatants were collected from each well and cytokine concentrations were determined by commercially available ELISA kits purchased from Endogen (IL-4, IL-10, and IL-12; Woburn, MA), Medgenix Diagnostics IL-6; Fleurus, Belgium), and BioSource International (IFN-γ and TGF-β; Fleurus, Belgium).

Statistical analysis
The existence of statistically significant differences between mean cytokine concentrations was analyzed by the Mann-Whitney U test for nonparametric values using GraphPad Prism Version 2 software (GraphPad, San Diego, CA).

Results

CD8⁺ Ts phenotype
The phenotype of CD8⁺ Ts was analyzed in all subjects included in the study. The phenotypic analysis of CD8⁺ Ts showed that the expression of most Ags tested did not differ among SLE patients or between SLE patients and healthy subjects. In fact, both percentages of positive cells and intensity of Ag expression (as evaluated by analysis of the linear mean fluorescence) were comparable. However, the percentage of positive cells expressing LFA-1 Ag and P58.2, P70, and CD94 NK receptors was moderately decreased in some SLE patients with respect to healthy controls. This finding was not consistent in all SLE patients, and no statistical significant differences were found among groups. The phenotype of CD8⁺ Ts from three subjects representative of the studied population is shown in Table II.

CD8⁺ Ts inhibition of anti-CD3 mAb-induced PBMC proliferation
To assess the suppressor capacity of CD8⁺ Ts generated from SLE patients, we evaluated their ability to inhibit PBMC proliferation induced by the anti-CD3 mAb UCHT-1. CD8⁺ Ts from patients with SLE in remission and from healthy donors showed a comparable suppressor activity which was related to the number of CD8⁺ Ts added to cultures (Fig. 1). Moreover, the suppressor activity of CD8⁺ Ts from SLE patients in remission and from healthy donors assessed in two assays performed at 3-mo intervals using two separate preparations of CD8⁺ Ts was stable (data not shown). On the contrary, CD8⁺ Ts lymphocytes generated from patients with active SLE did not show any inhibitory activity even when tested at high concentrations (8 × 10⁵ cells; Fig. 1). The lack of suppressor activity was clearly related with the active state of the disease. Indeed, it was possible to generate CD8⁺ Ts cells with inhibitory capacity following response to treatment (Fig. 2).

CD8⁺ Ts suppressive function is mediated by soluble factors
The inhibitory activities of CD8⁺ Ts cocultured with anti-CD3 mAb-stimulated PBMC or cultured with the latter cells in a transwell system were compared. The extent of suppression was similar in both experimental models (data not shown), thus supporting the hypothesis that the inhibitory activity might be mediated by soluble factors. Accordingly, the adding of an anti-IFN-γ mAb to the cultures inhibited the CD8⁺ Ts function (Table III). Furthermore, preincubation of CD8⁺ Ts with the oligonucleotide antisense of the IL-6 genetic sequence counteracted their inhibitory activity (Fig. 3). In the shown experiment, the percent inhibition of PBMC proliferation by CD8⁺ Ts was 60% (E) but it became <2% when CD8⁺ Ts were preincubated with the antisense oligonucleotide (I) and 16% when both CD8⁺ Ts and monocytes were preincubated.

Table III: CD8⁺ Ts cell inhibition of PBMC proliferation is blocked by anti-IFN-γ mAb

<table>
<thead>
<tr>
<th>Culture Conditions</th>
<th>PBMC</th>
<th>Anti-CD3 mAb UCHT-1</th>
<th>CD8⁺ Ts</th>
<th>Anti-IFN-γ mAb 69</th>
<th>cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>182 ± 48</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>4230 ± 256</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>1680 ± 312</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>4280 ± 352</td>
</tr>
</tbody>
</table>

* PBMC proliferation induced by an anti-CD3 mAb was evaluated culturing the cells in the presence or not of CD8⁺ Ts. In one culture condition, the anti-IFN-γ mAb 69 (10 μg/ml) was coincubated with PBMC and CD8⁺ Ts. Shown are the results of a representative experiment performed with PBMC from a healthy donor. Comparable results were obtained using PBMC from three healthy donors and three patients with SLE in remission. Values are expressed as mean ± SD of triplicate wells.
FIGURE 3. Effects of oligonucleotides sense and antisense of IL-6 genetic sequence on CD8\(^+\) Ts inhibitory activity of anti-CD3 mAb-induced PBMC proliferation. The results are expressed in cpm ± SD of triplicates. A, Irradiated macrophages alone; B, PBMC alone; C, irradiated macrophages plus nonstimulated PBMC; D, irradiated macrophages plus anti-CD3 mAb-stimulated PBMC; E, as D plus autologous CD8\(^+\) T cells (80,000 cells/well); F, as E but macrophages were preincubated with the sense oligonucleotide; G, E but macrophages were preincubated with the antisense oligonucleotide; H, as E but CD8\(^+\) Ts were preincubated with the sense oligonucleotide; I, as E but CD8\(^+\) Ts were preincubated with the antisense oligonucleotide; L, as E but both macrophages and CD8\(^+\) Ts were preincubated with the sense oligonucleotide; and M, as E but both macrophages and CD8\(^+\) Ts were preincubated with the antisense oligonucleotide.

with the antisense oligonucleotide (M). Interestingly, counteraction of inhibitory activity did not occur when monocytes were exposed to the antisense oligonucleotide (G), thus suggesting that in this culture the main font of IL-6 is constituted by CD8\(^+\) Ts and not by monocytes. The sense oligonucleotide (used as control) did not exert any effect on the inhibitory function of CD8\(^+\) Ts. The shown experiment was performed with cells from a healthy donor: comparable results were obtained when cells from SLE patients in remission were used (data not shown).

The cytotoxic and apoptosis inducing activities of CD8\(^+\) Ts cells were also investigated. The results clearly demonstrate that CD8\(^+\) Ts did not induce any cytotoxic and/or apoptotic effect on target cells (Table IV).

**CD8\(^+\) Ts cytokine secretion pattern**

To define the secretion pattern of CD8\(^+\) Ts in healthy donors and in SLE patients, the concentrations of IL-4, IL-6, IL-10, IL-12, IFN-\(\gamma\), and TGF-\(\beta\) were determined in the supernatants of CD8\(^+\) Ts cultures activated with IL-2 or anti-CD3 mAb UCHT-1. The concentration of IL-6 was significantly decreased in cultures from SLE patients with respect to controls (Fig. 4). By contrast, the concentration of IL-12 in nonstimulated and IL-2-stimulated cultures from patients with active SLE was significantly increased with respect to healthy subjects and SLE patients in remission (Fig. 4). No significant differences were observed in IL-4, IL-10, IFN-\(\gamma\), and TGF-\(\beta\)-concentrations between SLE patients and healthy subjects (data not shown).

To further assess that CD8\(^+\) Ts were the source of IFN-\(\gamma\), IL-6, and IL-12 in our cultures, we performed an intracellular staining using specific mAbs. The analysis clearly demonstrated the presence of all cytokines in CD8\(^+\) Ts (Fig. 5).

**Opposite effects of IL-6 and IL-12 on anti-CD3 mAb-induced PBMC proliferation**

The finding that IL-6 level was decreased in the supernatant of CD8\(^+\) Ts cultures from all SLE patients, whereas the IL-12 level was increased in the supernatant of CD8\(^+\) Ts cultures from SLE patients with active disease alone prompted us to analyze the effect of these cytokines on the anti-CD3 mAb UCHT-1-stimulated PBMC proliferation. As expected, IL-6 and IL-12 induced a dose-dependent inhibition and stimulation of PBMC proliferation, respectively (Table V).

**Discussion**

The results of this study show that CD8\(^+\) Ts cells can be generated from healthy donors and from patients with SLE in remission, but not from patients with active SLE. In particular, the suppressor activity of CD8\(^+\) Ts seems mainly attributable to the secretion of specific cytokines (IFN-\(\gamma\), IL6) rather than to cell-cell interactions. CD8\(^+\) Ts cells from SLE patients are characterized by a peculiar cytokine secretion pattern that may change in the course of SLE relapses.

Several abnormalities of cellular immunity have been described in SLE (14). Among them, the lack of suppressor T cell activity may play a role in the pathogenesis of disease (6). Nevertheless,
the existence and relevance of CD8$^+$ T suppressor lymphocytes is still a debated issue (15). T cells with suppressor function were first described by Gershon and Kondo in 1971 (16). Thereafter, several observations supported their role in immune regulation processes. In fact, at least two lines of evidence suggest the existence of CD8$^+$ T cells able to inhibit immune responses. First, the isolation of Ag-specific and alloantigen-specific T suppressor lymphocytes from mice and humans (17–20) and, second, the identification and functional characterization of CD8$^+$ T lymphocytes which suppress autoimmune responses in mice with experimental autoimmune encephalomyelitis (21).

The results of the present work indicate that CD8$^+$ Ts can be generated from SLE patients in remission but not from SLE patients with active disease. However, it was possible to generate CD8$^+$ Ts from SLE patients with active disease who attained clinical and laboratory remission following aggressive immunosuppressive treatment. Thus, the impairment of CD8$^+$ Ts function appears to be reversible, suggesting that this suppressor cell population (or its precursors) still exists during SLE relapses although in an inactive state. Different sets of experiments (performed in transwell system or using biological agents neutralizing IFN-$\gamma$ or IL-6) strongly suggested that the CD8$^+$ Ts activity is mediated by soluble factors. The studies were performed culturing CD8$^+$ Ts in the presence of autologous macrophages because these latter cells are needed to induce CD8$^+$ T lymphocyte suppressor function (22). However, macrophage cytokine secretion likely had a poor direct effect in determining inhibition of proliferation as suggested by the fact that counteraction of inhibition was possible only when CD8$^+$ Ts, but not macrophages, were preincubated with the oligonucleotide antisense of the IL-6 genetic sequence. To explain the different biological behaviors among CD8$^+$ Ts from healthy donors, SLE patients in remission, and SLE patients with active disease, the cytokine secretion pattern of these cells was characterized. In particular, the secretion of IL-6 and IL-12, which inhibit and stimulate, respectively, the anti-CD3 mAb-induced PBMC proliferation, was analyzed. A different cytokine secretion pattern was identified in CD8$^+$ Ts from SLE patients and healthy donors. CD8$^+$ Ts from all SLE patients secreted lower amounts of IL-6 as compared with healthy donors. In contrast, CD8$^+$ Ts from SLE patients with active disease secreted, both in nonstimulated and IL-2-stimulated conditions, higher amounts of IL-12 as compared with SLE patients in remission and healthy donors. All together these findings suggest that IFN-$\gamma$ and IL-6 are both required to obtain the CD8$^+$ Ts-mediated inhibition of PBMC proliferation. In fact, when each of these cytokines was singularly neutralized, a counteraction of the CD8$^+$ Ts inhibitory activity occurred. It is likely that even low secretion levels of such cytokines are sufficient to mediate the CD8$^+$ Ts function. In fact, patients with SLE in...
remission showed conserved CD8+ Ts inhibitory activity (although in the presence of reduced secretion of IL-6 by this cell subpopulation) that was counteracted by the oligonucleotide antisense of the IL-6 genetic sequence as in the case of CD8+ Ts from healthy subjects. Furthermore, these data suggest that the increased secretion of a strong stimulatory cytokine such as IL-12, as in the case of patients with active SLE, is able to overcome the inhibitory signals mediated by both IFN-γ and IL-6. Thus, it can be hypothesized that CD8+ Ts from SLE patients may have an unbalanced secretion of inhibitory (IL-6) and stimulatory (IL-12) cytokines. When the secretion of stimulatory cytokine(s) is increased, the inhibitory function of CD8+ Ts may be impaired. It is conceivable that this alteration in the secretion pattern of CD8+ Ts cells from SLE patients might derive from the multiple abnormalities of the intracellular signal transduction pathways that have been described in lymphocytes from SLE patients (22). The secretion of IL-4, IL-10, IFN-γ, and TGF-β by CD8+ Ts from SLE patients was also determined because it has been reported that these cytokines may mediate the suppressor function of CD8+ T lymphocytes (16, 23). No differences between SLE patients and healthy subjects were observed. Finally, no significant differences in cytotoxic activity and apoptosis induction were observed between CD8+ Ts cells from SLE patients and healthy subjects.

With regard to the CD8+ Ts phenotype, moderate variation of LFA-1 and PS8.2, P70, and CD94 NK receptor percentage expression was found only in some SLE patients with respect to controls. However, the functional behavior of CD8+ Ts was comparable within each group of subjects and independent from these sporadic phenotypic variations. Thus, it appears unlikely that the observed phenotypic diversities could derive from different cell activation states and be responsible for the different functional activities among groups. Interestingly, CD28 Ag was found to be poorly expressed on these cells. This is in agreement with the already described phenotype of CD8+ suppressor cells (20) and perhaps suggests that these cells can use alternative cell membrane mediator(s) of activation.

In conclusion, we demonstrate that the functional activity of CD8+ Ts is impaired in SLE patients with active disease and that this alteration could be related to the abnormal cytokine secretion pattern of these cells. Our results apparently disagree with previous literature data reporting that raised levels of IL-6 are found in serum from SLE patients and that the secretion of IL-12 by mononuclear cells from SLE patients is decreased with respect to healthy donors (24, 25). We hypothesize that the activation of CD8+ T suppressor lymphocytes would take place at the site in which immune responses are developing. Therefore, secreted cytokine(s) would regulate CD8+ Ts function locally and should not induce systemic effects. Moreover, the impairment of CD8+ Ts function in SLE patients with active disease could lead to the activation of CD4+ Th2 cells, thus explaining the cytokine pattern found in serum. Further investigations will better clarify the relevance in vivo of our findings mainly addressing the point whether the observed deficiency of CD8+ Ts function in SLE patients with active disease has a pathogenic role or whether it is only consequence of other primitive alterations of the immune system homeostasis.

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References