CONCISE REPORT

Cyclosporin-A Is Selectively Cytotoxic to Human Leukemic T Cells In Vitro

By Thomas H. Tötterman, Antero Danersund, Kenneth Nilsson, and Andreas Killander

We have studied the antileukemic effects of cyclosporin-A (CyA) on the cells of a number of human hematopoietic biopsies and established cell lines in vitro. CyA killed the tumor cells but not the nonmalignant control cells from three of four patients with T-lymphocytic leukemia/lymphoma at concentrations comparable to those recommended in clinical long-term immunosuppressive therapy. The leukemic cells from 7 patients with B-type chronic lymphocytic leukemia were insensitive to CyA. The drug was cytostatic and cytolytic to three of five T-lymphoblastic leukemia cell lines tested. With the possible exception of a plasma cell line, all other control cell lines (B-lymphocytic lymphoma, histiocytic lymphoma, fibroblast, and glia cell lines) were resistant to CyA. These observations indicate that CyA or its derivatives may be useful as a highly selective antitumor agent in different T-cell malignancies.

MATERIALS AND METHODS

Patients

Freshly isolated peripheral blood (or pleural effusion) cells from 11 patients with lymphoid leukemia/lymphoma were analyzed for CyA sensitivity in vitro (Table 1). The patients with T-cell malignancies included one case of Sézary's syndrome (SS; diagnosis based on typical cerebriform lymphocyte nuclei, lymph node pathology, skin symptoms, and surface marker profile), one case of highly differentiated T-lymphocytic lymphoma presenting with leukemia (T-LL; diagnosis by bone marrow, lymph node, and spleen morphology and surface markers), one case of T-lymphoblastic lymphoma (T-LBL; diagnosed by pleural effusion cell and lymph node morphology plus surface markers), and one case of chronic T-lymphocytic leukemia (T-CLL). The leukemic B cells tested were obtained from seven patients with chronic B-lymphocytic leukemia (diagnosis of T/B-CLL based on marrow and lymph node morphology and surface markers). None of the patients had received cytostatic treatment within 3 wk prior to study.

Preparation of Leukemic Blood Cells

Leukemic cells were isolated from heparinized blood samples by Ficoll-Isopaque (Pharmacia, Uppsala, Sweden) density separation. In the SS patient, the isolated lymphocytes were further rosetted with aminoethyl-isothiouronium bromide-treated sheep red cells (AET-SRBC) at 4°C. After centrifugation on Ficoll-Isopaque, the rosetted (SS-T) and nonrosetted (SS-NT) lymphocyte fractions were collected at the bottom and interface of the gradient, respectively. The different biopsy cells were washed 3 times in culture medium, checked for viability (always >87%; trypan blue exclusion), and characterized by surface markers before use in CyA tests.

Established Cell Lines

The surface marker characteristics of the tested hematopoietic cell lines and selected references are given in Table 1. The SS-LCL is...
an unpublished Epstein-Barr virus (EBV) carrying lymphoblastoid B-cell line established spontaneously from non-neoplastic blood B cells of our Sézary patient. The nonmalignant human glia cell line 787CG2 and a human fibroblast line U-1523 (Dr. B. Westermark, unpublished) were also tested for CyA sensitivity.

**Surface Markers**

The following surface markers were employed: T cells were characterized by rosette formation with AET-SRBC (panmature T) and immunofluorescence tests with the monoclonal mouse hybridoma antibodies OKT-3 (panmature T), OKT-4 (helper/inducer T subset), and OKT-8 (suppressor/cytotoxic T subset). The surface immunoglobulin (sIg) of B cells was characterized by fluorescein-conjugated F(ab')2 rabbit antibodies (Kallestad, Chaska, Minn.) to human heavy (polyvalent) and light Ig chains. Monocytes were identified by the monoclonal mouse antibody Mo 1 (polyvalent) and HLA-DR-positive cells were stained using a mouse monoclonal antibody.11 The OKT and OKM antibodies were purchased from Ortho Diagnostics (Raritan, N.J.) and the anti-HLA-DR from New England Nuclear (Boston, Mass.). The second layer antibody was a specific fluorescein-labeled sheep anti-mouse Ig serum (State Bacteriology Lab., Stockholm, Sweden) showing no background staining of CLL or normal buffy coat cells. The rosetting techniques for the quantitation of IgG-Fc and C3 receptors have been detailed.12

**Tests for CyA Sensitivity**

CyA was kindly provided by Dr. J. F. Borel, Sandoz, Basel. A stock solution was prepared by dissolving CyA in absolute ethanol (1 mg/ml).

For tests of CyA sensitivity, cells of established cell lines were harvested from logarithmically growing stock cultures and incubated in duplicate test cultures at an initial cell density of 3 x 10^5 cells/ml to allow logarithmic growth throughout the whole test period of 72 hr. Leukemic biopsy cells were incubated at a density of 5-6 x 10^5 cells/ml. The medium, F-10, was supplemented with 10% fetal calf serum and antibiotics (100 IU/ml of penicillin, 50 µg/ml of streptomycin). The effect of CyA (0.1-10 µg/ml) was assessed by daily determinations of viability by trypan blue exclusion and cell concentration by an electronic cell counter. In control experiments, no cytotoxic/cytostatic effects were registered after addition of (0.01%-0.15%) ethanol only. The rate of DNA synthesis was determined by adding 1 µCi of tritiated thymidine (Amersham, England) to 5 x 10^5 viable cells in 1 ml of medium and counting the radioactivity incorporated after 1 hr.

**RESULTS**

**Surface Marker Profiles of Cells Tested**

Table 1 summarizes the surface marker characteristics of the different cell types tested for CyA sensitivity. The SS-T cells and T-CLL cells represented monoclonal helper T lymphocytes, whereas SS-NT and SS-LCL cells consisted of polyclonal nonmalignant B cells. The T-LL cells carried the suppressor T cell and OKM antigens, whereas T-LBL cells were positive for both inducer and suppressor markers. The seven B-CLL clones all expressed monoclonal surface Ig. The T-cell lines SKW-3 and CCRF-CEM were of the helper T type, whereas Molt-4 and T-45 cells stained for T subset characteristics in our hands, but mainly for suppressor T antigen. The JM cells were helper T type, whereas Molt-4 and T-45 cells stained for T subset characteristics in our hands, but mainly for suppressor T antigen. The JM cells were negative for T subset characteristics in our hands, but formed SRBC rosettes.

**CyA Sensitivity of Leukemic Biopsy Cells**

The leukemic SS-T cells from the patient with Sézary's syndrome were highly sensitive for the cyto-
toxic effect of CyA in a dose- and time-dependent manner (Fig. 1). However, the nonmalignant SS-NT (mainly B cells) from the same patient were completely resistant to CyA (Fig. 2), as were the derived lymphoblastoid B line cells (SS-LCL) (Fig. 3). Figure 4 shows that the T-LL lymphoma cells were highly sensitive to CyA in low concentrations, whereas T-LBL cells were moderately sensitive. The T-CLL cells were, however, resistant to the drug. The leukemic biopsy cells from seven patients with B-CLL were not killed by CyA (Table 2). Because of the low proliferative capacity of all different biopsy cells, the effect of CyA on DNA synthesis was not analyzed.

Table 2 shows that some T-cell lines, namely Molt-4, SKW-3, and to a lesser extent JM, were killed by intermediate to high concentrations of CyA. This was accompanied by a decrease in the rate of DNA synthesis of viable cells (Table 2). The sensitivity of T lines to CyA was neither correlated to the helper/suppressor-cytotoxic T-cell phenotype nor to HLA-DR expression. The control myeloma cell line U-266 was sensitive to the highest CyA concentrations, whereas the B-lymphocytic lymphoma line (U-698) and the histiocytic lymphoma cell line (U-937) were insensitive. The glia cell line 787 CG and the fibroblast line 1523 were completely resistant to CyA (data not shown).

**Fig. 1.** Sensitivity of purified leukemic Sézary T (SS-T) cells to different concentrations (0.1–10.0 μg/ml) of CyA in vitro. Co, control cultures.

**Fig. 2.** Sensitivity of (nonmalignant) non-T-cells (SS-NT) from the Sézary patient to 0.1–10.0 μg/ml of CyA. Co, control cultures.

**Fig. 3.** Sensitivity of (nonmalignant) B-lymphoblastoid cell line cells (SS-LCL) from the Sézary patient to 1.0–10.0 μg/ml of CyA. Co, control cultures.

**Fig. 4.** Cytotoxic effect of CyA (indicated concentrations in μg/ml; Co, control cultures) to various T leukemia/lymphoma biopsy cells. (○--○) T-type chronic lymphocytic leukemia (T-CLL) cells; (●--●) T-lymphocytic lymphoma (T-LL) cells; (□--□) T-lymphoblastic lymphoma (T-LBL) cells.
Table 2. Effect of Cyclosporin-A on the Viability, Growth, and DNA Synthesis of Different Cell Lines and B-CLL Cells

<table>
<thead>
<tr>
<th>Cells</th>
<th>Day 0*</th>
<th>Cyclosporin (μg/ml)†</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>T-cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SKW-3</td>
<td>100</td>
<td>+27(100)</td>
<td>+33(±166)</td>
</tr>
<tr>
<td>Molt-4</td>
<td>100</td>
<td>+10(100)</td>
<td>+26(±40)</td>
</tr>
<tr>
<td>JM</td>
<td>100</td>
<td>+12(100)</td>
<td>+10(−12)</td>
</tr>
<tr>
<td>T-45</td>
<td>100</td>
<td>+41(100)</td>
<td>+36(±0)</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>100</td>
<td>+52</td>
<td>+43</td>
</tr>
<tr>
<td>Plasma cell line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-266</td>
<td>100</td>
<td>+63</td>
<td>+25</td>
</tr>
<tr>
<td>B-lymphocytic lymphoma line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-698</td>
<td>100</td>
<td>+116</td>
<td>+150</td>
</tr>
<tr>
<td>Histiocytic lymphoma line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-937</td>
<td>100</td>
<td>−10</td>
<td>+30</td>
</tr>
<tr>
<td>CLL biopsy cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>100</td>
<td>+2</td>
<td>−23</td>
</tr>
<tr>
<td>Patient 2</td>
<td>100</td>
<td>±0</td>
<td>±0</td>
</tr>
<tr>
<td>Patient 3</td>
<td>100</td>
<td>+6</td>
<td>−19</td>
</tr>
<tr>
<td>Patient 4</td>
<td>100</td>
<td>−36</td>
<td>−36</td>
</tr>
<tr>
<td>Patient 5</td>
<td>100</td>
<td>−24</td>
<td>−32</td>
</tr>
<tr>
<td>Patient 6</td>
<td>100</td>
<td>−10</td>
<td>−10</td>
</tr>
<tr>
<td>Patient 7</td>
<td>100</td>
<td>−44</td>
<td>−44</td>
</tr>
</tbody>
</table>

*100% = viability (%) x number of plated cells (0.3–0.5 x 10^6)/ml of culture medium.
†Figures in brackets are relative change (%) in the rate of DNA synthesis of 5 x 10^6 viable cells in CyA-treated cultures compared with control cultures (=100%).
‡No additions to culture. In control experiments, no effect was registered using the solvent, ethanol, per se (data not shown).

DISCUSSION

The results presented here demonstrate that the leukemic cells of many patients with T-cell but not B-cell malignancies may be highly sensitive to CyA in vitro. Importantly, CyA killed malignant T cells at concentrations comparable with the serum levels (0.1–3 μg/ml) measured during continuous immunosuppression after cadaver kidney transplantation in man.\(^7\) The T-cell selectivity of the cytostatic/cytolytic effects of CyA was further confirmed when investigating different leukemia/lymphoma lines. Significantly, CyA did not affect the nonleukemic biopsy cells nor the established EBV-carrying B-lymphoblastoid cell line from the patient with Sézary's syndrome but was specifically cytotoxic for her leukemic T cells. This indicates that CyA may be used for specific antileukemic therapy in Sézary patients and those with related T-cell disorders. However, a prescreening of CyA sensitivity in vitro seems necessary because of the variable effects on different malignant T-cell clones observed in this study.

CyA has been reported to exert its immunosuppressive effects more or less specifically on a given subset of normal T cells. Some investigators showed an inhibition of the release of IL-2 from antigen-triggered helper (OKT4\(^+\)) T cells together with a reduced IL-1 secretion from HLA-DR-positive antigen-presenting cell types.\(^10,11\) Recently, Palacios and Möller\(^10,33\) observed that CyA may also bind to receptors for HLA-DR antigens and OKT-3 antibody on T cells. This could render the T cells unresponsive to IL-2. Paavonen and Häyry\(^33\) demonstrated comparable inhibitory effects of CyA on the DNA synthesis of pokeweed-mitogen-stimulated T\(\gamma\) and T\(\mu\) blasts, as well as B blasts. In the present study, we did not observe any clear-cut association between the CyA sensitivity of T cells and the expression of OKT-4 or OKT-8 phenotype. CyA seems to interact with OKT-3 complementary structures on T cells.\(^3\) The OKT-3 receptor could, theoretically, be a "permissive" target structure for the cytostatic/cytolytic action of CyA on malignant T leukemia/lymphoma cells, as most of the killed cell types expressed this receptor.

In conclusion, CyA seems to be selectively cytotoxic and cytostatic to several types of leukemic biopsy T cells and T-cell lines but not other cell types. These effects are seen with concentrations of the drug corresponding to those used in long-term clinical immunosuppression.
REFERENCES

5. Calne RY, Rolles K, White DJG, Thiru S: Cyclosporin A initially as the only immunosuppressant in 34 recipients of cadaveric organs: 32 kidneys, 2 pancreases and 2 livers. Lancet 2:1033, 1979
Cyclosporin-A is selectively cytotoxic to human leukemic T cells in vitro

TH Totterman, A Danersund, K Nilsson and A Killander