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# STIMULATION OF LYMPHOKINE RELEASE FROM T LYMPHOBLASTS

## Requirement for mRNA Synthesis and Inhibition by Cyclosporin A

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T cells secrete a variety of hormone-like proteins or lymphokines that mediate many aspects of the immune response. Among these lymphokines are T cell growth factor or interleukin 2 (IL-2);<sup>1</sup> the macrophage-activating factor, immune or  $\gamma$ -interferon; and a number of B cell-stimulating factors (1-3). A standard means for inducing the release of lymphokines is to stimulate rodent spleen cells with concanavalin A (Con A). Lymphokine release occurs over a 24-48 h period. By 72 h, the cultures consist of IL-2-responsive T lymphoblasts that no longer release lymphokine (4).

In this paper we studied the capacity of Con A-induced T blasts to produce lymphokines as a model for the behavior of sensitized T cells in a cell-mediated immune response. IL-2, immune interferon, B cell-stimulating factors, and factors enhancing the development of cytotoxic T cells were all quickly released over a 5-20 h period after the readdition of lectin to T blasts. Lymphokine secretion required the induction of new lymphokine-specific mRNA and new protein synthesis. We also studied the effects of cyclosporin A (CSA) in this model. This immunosuppressive drug is known to act early in several primary responses (5-9), and was recently shown to block the release of IL-2 from antigen-specific T cell blasts (10). We found that CSA blocks the production of many lymphokines and that this block is at the level of biologically active, lymphokine-specific mRNA.

### Materials and Methods

**Animals.** BALB/c mice were purchased from Charles River Breeding Laboratories, Inc., Wilmington, MA, and NCS mice were bred at The Rockefeller University. *Xenopus laevis* frogs were obtained from the South African Snake Farm, Fish Hoek 7973, South Africa.

**Reagents.** Con A three times crystallized was purchased from Miles Laboratories, Inc., Elkart, IN; 4- $\alpha$ -phorbol 12-myristate 13-acetate (PMA) and cycloheximide from Sigma

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<sup>1</sup> **Abbreviations used in this paper:** Con A, concanavalin A; CSA, cyclosporin A; CTL, cytolytic T lymphocytes; [<sup>3</sup>H]TdR, tritiated thymidine; IFN, interferon; IL-2, interleukin 2; MEM, minimum essential medium; PFC, plaque-forming cell; PHA, phytohemagglutinin; PMA, 4- $\alpha$ -phorbol 12-myristate 13-acetate; SDS, sodium dodecyl sulfate.

Chemical Co., St. Louis, MO; oligo(dT) cellulose type 77 from P-L Biochemicals, Inc., Milwaukee, WI; tissue culture plasticware from Falcon Labware, Oxnard, CA; [ $^3\text{H}$ ]-thymidine ([ $^3\text{H}$ ]TdR) (sp act, 17.5 Ci/mM), [ $^{35}\text{S}$ ]methionine (sp act, 1,000 Ci/mM), and  $^{51}\text{Cr}$  (sp act, 331 mCi/mg) from Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, NY. CSA was a generous gift from Dr. J. F. Borel, Sandoz Ltd., Basel, Switzerland. A stock solution of CSA was made in dimethyl sulfoxide (3 mg/ml) and diluted in minimum essential medium (MEM) before each use.

**Stimulation of Lymphokine Release.** Con A-activated spleen cells were prepared as described elsewhere (11). Briefly, dissociated spleen cells were plated at  $1.5 \times 10^6$  cells/ml in MEM containing 20  $\mu\text{g}/\text{ml}$  gentamycin, 10% heat-inactivated fetal calf serum,  $5 \times 10^{-5}$  M 2-mercaptoethanol, and 1  $\mu\text{g}/\text{ml}$  Con A. After 3 d, most of the viable cells were lymphoblasts which were then collected, washed, and plated at  $2 \times 10^6$  cells in 35-mm plates. The medium was 1 ml serum-free MEM containing  $5 \times 10^{-5}$  M 2-mercaptoethanol and 5  $\mu\text{g}/\text{ml}$  Con A; to this was added 1  $\mu\text{g}/\text{ml}$  CSA or 5  $\mu\text{g}/\text{ml}$  cycloheximide, or, as a control, solvent only. After 2 h, the Con A-induced cell monolayers were washed to remove Con A, CSA, or cycloheximide, and 2 ml of MEM was added. At the desired time, supernatants for the bioassay of IL-2 or other lymphokines (see below) were harvested. IL-2 was also assayed in cells that were lysed by three cycles of freezing and thawing. All samples were stored at  $-20^\circ\text{C}$  prior to bioassay. EL-4 cells ( $5 \times 10^5$  cells/ml) were stimulated with PMA (10 ng/ml) in the presence or absence of CSA (1  $\mu\text{g}/\text{ml}$ ) again in serum-free medium to allow cell attachment and washing. After 2 h, cells were washed to remove PMA and CSA and were resuspended at the same concentration in fresh medium. At the desired time, supernatants and cells were harvested. Samples were stored at  $-20^\circ\text{C}$  before the bioassay for lymphokine activity.

**Isolation of Poly(A) $^+$  RNA.** Total RNA was extracted from stimulated spleen lymphoblasts ( $3\text{--}5 \times 10^6$ ) and from EL-4 cells ( $3\text{--}5 \times 10^8$ ) using a guanidinium/cesium chloride method (12, 13). Lymphoblasts were plated at  $2 \times 10^6$  cells/ml (40 ml in 150-mm culture plates) in MEM to which Con A (5  $\mu\text{g}/\text{ml}$ ) and/or CSA (1  $\mu\text{g}/\text{ml}$ ) was added. After 5–7 h incubation, cells were collected and extracted. For the preparation of RNA from EL-4, cultured cells ( $5 \times 10^5$  cells/ml) were stimulated for 4 or 7 h with PMA (10 ng/ml) plus or minus CSA (1  $\mu\text{g}/\text{ml}$ ), collected, and extracted. The preparation of poly(A) $^+$  RNA was accomplished by batchwise fractionation using oligo(dT) cellulose. Total RNA was diluted in  $\text{H}_2\text{O}$  to 100 OD $_{260}$  U/ml, heated at  $60^\circ\text{C}$  for 2 min, and cooled quickly. An equal volume of 2 $\times$  HSB buffer (10 mM Tris HCl, pH 7.4, 0.12 M NaCl, 1 mM EDTA, 0.2% sodium dodecyl sulfate [SDS]) and prewashed oligo(dT) cellulose (1 mg cellulose for 1 OD $_{260}$  unit) were added. After the solution was shaken for 1 h at room temperature, the resin was washed six times with HSB and twice with HSB minus SDS, and then poly(A) $^+$  RNA was eluted three times with  $\text{H}_2\text{O}$ . For each wash, the resin was collected by centrifugation at 3,000 rpm for 3 min and the eluate was precipitated with 3 vol of alcohol after adjustment to 0.3 M NaCl. The precipitated RNA was dissolved in  $\text{H}_2\text{O}$  and stored at  $-80^\circ\text{C}$ . 100–120  $\mu\text{g}$  of poly(A) $^+$  RNA was obtained from  $3 \times 10^8$  cells.

**Translation of Poly(A) $^+$  RNA.** 40 ng of poly(A) $^+$  were injected in *Xenopus laevis* oocytes. Groups of 20 injected oocytes were incubated in microwells containing 200  $\mu\text{l}$  of Barth's incubation medium (14) supplemented with bovine serum albumin (0.5 mg/ml) and Trasylol (50 U/ml). After 24-h intervals the medium was collected and centrifuged for 5 min in an Eppendorf microcentrifuge, and aliquots of supernatants were assayed for lymphokine activity. Cell-free translation of poly(A) $^+$  RNA was performed using a wheat germ system according to Erikson and Blobel (15).

**Biosynthetic Labeling of Lymphoblastoid Cells.**  $2 \times 10^6$  blast cells were incubated for 2 h with Con A (5  $\mu\text{g}/\text{ml}$ ) and/or CSA (1  $\mu\text{g}/\text{ml}$ ) in 1 ml serum-free medium and the supernatant was removed. 1 ml of MEM containing 1/20th of the usual level of methionine and 50  $\mu\text{Ci}/\text{ml}$  [ $^{35}\text{S}$ ]methionine (1,000 Ci/mmol) was added and the incubation continued for a further 20 h. 100  $\mu\text{l}$  of the resulting supernatant was mixed with sample buffer and applied to a 12.5% SDS-polyacrylamide gel according to Laemmli (16).

**IL-2 Assay.** IL-2 activity in conditioned medium and cell lysates were assayed on 3-d Con A-stimulated spleen cells as described previously (11). Serial dilutions of sample to

be assayed were prepared in microtest wells (50  $\mu$ l) to which 50  $\mu$ l of a blast suspension ( $4 \times 10^5$  cells/ml) plus 20 mM  $\alpha$ -methyl mannoside were added. After 20 h incubation, microwells were pulsed with 1.7  $\mu$ Ci of [ $^3$ H]TdR. The cultures were harvested 4 h later with a cell harvester, and filters were counted in Liquifluor-Toluene. The [ $^3$ H]TdR uptakes reported are from the linear portion of the dose response curve.

**Interferon (IFN) Assay.** IFN activity in conditioned medium was determined by the inhibition of the cytopathic effect of vesicular stomatitis virus on the mouse fibroblast line L929/B. Confluent monolayers were seeded in microtest wells with threefold dilutions of the supernatant to be assayed. After 20 h of incubation, an infecting dose of virus was added. The plates were examined under the microscope to determine the last dilution of supernatants that inhibited the lysis of 50% of the cells. A standard for mouse IFN was included in each assay. CSA up to 5  $\mu$ g/ml did not inhibit the bioassay.

**B cell-stimulating Factors(s) Assay.** The assay for B cell helper factor(s) was done as described by Inaba et al. (17) using  $3 \times 10^6$  purified B cells as the responder and sheep red blood cells as antigen. In this assay the development of anti-sheep plaque-forming cells (PFC) required helper factor(s) and specific antigen. CSA inhibited the bioassay 50% at 10 ng/ml, so it was useful to use CSA-pulsed cells to study the inhibition of helper factor formation.

**Cytotoxic Differentiation Factor Assay.** Murine thymocytes develop antigen-specific cytotoxic T cells when cultured with antigen and Con A supernatant (18). We adapted this phenomenon to a more rapid, polyclonal assay.  $5 \times 10^5$  thymocytes from Swiss or B6.H-2k mice (4 wk old) were suspended in MEM supplemented with 10% heat-inactivated fetal calf serum,  $5 \times 10^{-5}$  M 2-mercaptoethanol, and 1  $\mu$ g/ml Con A, and cultured in microwells with several dilutions of conditioned media. The polyclonal cytotoxic T lymphocytes (CTL) response was measured after 48–72 h using  $^{51}$ Cr-labeled P815 as targets in the presence of 10  $\mu$ g/ml phytohemagglutinin (PHA). Con A supernatants were the positive control and mediated the development of Thy-1 $^+$ , Lyt-2 $^+$  lytic cells that required PHA to exhibit lysis. Purified (11) or recombinant IL-2 (Biogen, Cambridge MA), recombinant IFN (Genentech, San Francisco, CA), or IL-1 (produced by the J774 macrophage cell line stimulated with lipopolysaccharide), singly or together, did not support a CTL response in this assay.

## Results

**Cycloheximide Inhibits Synthesis of IL-2 from Mitogen-restimulated Blast Cells.** To study the production of lymphokine from sensitized T cells, we prepared lymphoblasts by stimulating mouse spleen cells for 72–96 h with the mitogen, Con A. Although these Con A blasts responded to IL-2, they did not produce this lymphokine (4). In contrast, restimulation with mitogen led to the production and secretion of high levels of IL-2 (Fig. 1). IL-2 activity in cell lysates and in the conditioned medium represented *de novo* synthesis because it was blocked by 5  $\mu$ g/ml cycloheximide (Fig. 1), an inhibitor of protein synthesis.

**CSA Inhibits Lymphokine Production from Con A-restimulated T Blasts.** Although CSA is regarded as an inhibitor of primary responses, CSA also inhibited the synthesis and release of IL-2 from lymphoblasts restimulated with Con A (Fig. 2A). In contrast, CSA did not block the proliferative response of T cells to exogenous IL-2 unless added at relatively high doses,  $>2$   $\mu$ g/ml (Fig. 2B).

Similar findings were made when the release of other lymphokines was evaluated. After the addition of Con A, control T lymphoblasts quickly produced immune IFN, B cell-stimulating factors, and mediators needed for the development of CTL (Table I). Release of all lymphokines was blocked by CSA. To rule out an inhibitory effect of residual CSA on the lymphokine bioassays, we pulsed the blasts rather than continuously exposing to CSA (Methods and Materials).

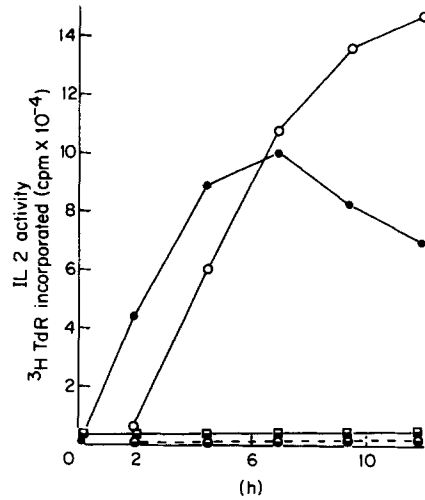


FIGURE 1. T lymphoblasts produce IL-2 in response to mitogen restimulation: effect of cycloheximide. 3-d Con A blasts were collected and adjusted to  $2 \times 10^6$  cell/ml in serum-free medium. 0.5 ml of cells were incubated for 2 h with or without Con A ( $5 \mu\text{g/ml}$ ), with or without cycloheximide ( $5 \mu\text{g/ml}$ ). After 2 h, medium was removed and replaced with 1 ml fresh medium. Supernatant and cells were collected at the indicated times and assayed for IL-2 activity. Control cultures: (○··○) conditioned medium, (●··●) cell lysates. Con A-restimulated cultures: (●··●) conditioned medium, (○··○) cell lysates. Con A + cycloheximide cultures: (□··□) conditioned medium, (■··■) cell lysates.

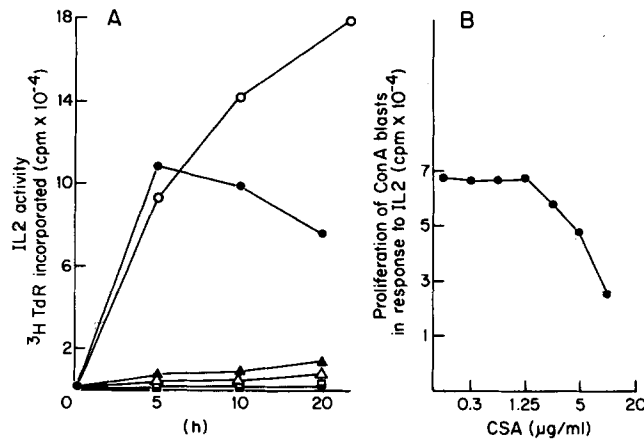


FIGURE 2. T lymphoblasts produce IL-2 in response to mitogen restimulation: effect of CSA. (A) 3-d Con A blasts were collected and adjusted at  $2 \times 10^6$  cell/ml in serum-free medium. 0.5 ml of cells were incubated for 2 h with Con A ( $5 \mu\text{g/ml}$ ) with or without CSA ( $1 \mu\text{g/ml}$ ) or left untreated. After 2 h, medium was removed and replaced with 1 ml fresh medium. Supernatants and cells were collected at the indicated times and assayed for IL-2 activity. Control cultures: (□) conditioned medium, (■) cell lysates. Con A-restimulated cultures: (○) conditioned medium, (●) cell lysates. Con A/CSA cultures: (△) conditioned medium, (▲) cell lysates. (B) 3-d blast cells were grown in the presence of a fixed amount of IL-2 and various concentrations of CSA. CSA concentrations  $> 2 \mu\text{g/ml}$  were required to block IL-2-mediated proliferation of activated T cells.

TABLE I  
*Effect of Cyclosporin A on Lymphokine Release by Con A-restimulated T Cell Blasts*

Lymphokine assayed	Source of supernatant and duration (h) of culture								
	Control			Con A			Con A + CSA		
	5	10	20	5	10	20	5	10	20
Interferon*	<4	<4	<4	30	100	300	<4	<4	<4
B cell-stimulating factor(s) <sup>‡</sup>	0	0	0	968	2,408	2,384	0	0	160
Cytotoxic differentiation factor(s) <sup>§</sup>	1.3	2.2	1.7	5.4	13	31.5	1.4	3	3.6

Conditioned medium (20%) was derived from blast cells that had been cultured for 5–20 h with or without Con A, with or without CSA.

\* Results are expressed as interferon units.

<sup>‡</sup> Results are expressed as PFC for individual cultures.

<sup>§</sup> Results are expressed as the percent <sup>51</sup>Cr released in a 4-h lectin-dependent cytotoxic assay.

No inhibition of the bioassays was noted when we mixed the conditioned medium from Con A- and Con A/CSA-treated cells.

*CSA Does Not Block Other Con A-mediated Responses.* Several experiments were then performed which suggested that the inhibitory effect of CSA on lymphokine production was selective. When total protein synthesis was studied in [<sup>35</sup>S]-methionine-labeled lymphoblasts, CSA did not alter the release of TCA-precipitable label or the pattern of released polypeptides, as detected by one-dimensional SDS-polyacrylamide gel electrophoresis (Fig. 3). Interestingly, the addition of Con A produced some changes in the polypeptides secreted by lymphoblasts; i.e., some bands were diminished and other bands induced when Con A was added, but none of these changes were altered by CSA (Fig. 3, arrows). CSA also did not inhibit polyclonal cytolytic T cell function, in which Con A-induced lymphoblasts killed <sup>51</sup>Cr-labeled targets in the presence of lectin (Table II). Lastly, CSA did not block the induction of plasminogen activator release in Con A-stimulated mouse macrophages (Table II). Together these results show that CSA inhibits the production of a variety of lymphokines from large T lymphocytes but does not affect the overall synthetic or killer activity of these cells, nor a Con A-induced response in macrophages.

*CSA Inhibits IL-2 Production and IL-2 mRNA Synthesis from the EL-4 Cell Line.* Previous work (19) had shown that the thymoma cell line EL-4 can be induced to produce IL-2 upon adequate stimulation. While exploring the effect of CSA on blast cells we observed that this drug also inhibited production of IL-2 from EL-4 cells that were challenged with the tumor promoter PMA (Table III). Unfortunately, our EL-4 cells did not release lymphokines other than IL-2.

The availability of large numbers of EL-4 cells allowed us to examine whether IL-2 mRNA was induced by PMA and if the induction of mRNA was sensitive to CSA. Indeed, when EL-4 cells were induced with PMA, active IL-2 mRNA could be extracted and translated in oocytes (Table IV), confirming the work of Bleackley et al. (20). When we injected mRNA from CSA-inhibited, PMA-treated EL-4 cells, no IL-2 was detectable in the medium conditioned by oocytes.

*Effect of CSA on the Induction of Biologically Active Lymphokine mRNA.* We

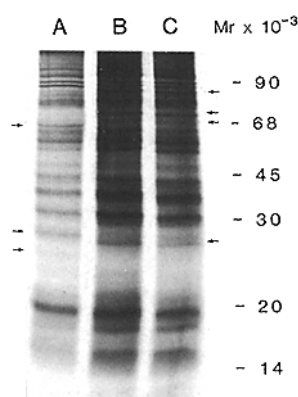


FIGURE 3. Autoradiogram of SDS-PAGE of biosynthetically labeled cultures of mitogen-restimulated T blasts. 3-d Con A blasts were collected and adjusted to  $2 \times 10^6$  cell/ml in serum-free medium. 0.5 ml cells were incubated for 2 h without Con A or with 5  $\mu\text{g/ml}$  Con A, or with Con A + 1  $\mu\text{g/ml}$  CSA. After 2 h, medium was removed and 1 ml medium containing [ $^{35}\text{S}$ ]methionine (50  $\mu\text{Ci/ml}$ ) was added to the cultures. After 18 h incubation, supernatants were analyzed in a 12.5% SDS-polyacrylamide gel. (A) Control cultures, (B) Con A-treated cultures, (C) Con A/CSA-treated cultures. Arrows indicate changes in secreted polypeptides that occur when Con A is added. This experiment was repeated using spleen cells that were depleted of B cells with anti-Ig and complement before Con A stimulation. Comparable results were obtained.

TABLE II  
Con A Responses That Are Not Inhibited by CSA

	-CSA		+CSA	
	-Con A	+Con A	-Con A	+Con A
Total protein synthesis*	20.424	35.241	19.430	32.137
Cytolytic activity <sup>†</sup>	3.1	48	2.8	49
Release of plasminogen activator <sup>‡</sup>	5	29.5	4.7	28.8

\* Blast cells ( $2 \times 10^6$ ) were restimulated with or without Con A, with or without CSA, in medium containing 50  $\mu\text{Ci/ml}$  [ $^{35}\text{S}$ ]methionine for 20 h. TCA-insoluble radioactivity was measured in aliquots of supernatants.

<sup>†</sup> Blast cells were tested for their capacity to lyse  $^{51}\text{Cr}$ -labeled P815 targets in a 4-h lectin (Con A)-dependent cytotoxic assay in the presence or absence of CSA (1  $\mu\text{g/ml}$ ).

<sup>‡</sup> Peritoneal macrophages ( $3 \times 10^6$ ) were treated with or without 5  $\mu\text{g/ml}$  Con A, with or without 1  $\mu\text{g/ml}$  CSA in serum-free medium for 20 h. Supernatants were collected and 25  $\mu\text{l}$  assayed for enzyme activity (27). Results are expressed as the percent of  $^{125}\text{I}$ -fibrin solubilized.

proceeded to look at the effects of CSA on the induction of lymphokine mRNA from primary T lymphoblasts. RNA was extracted from blast cells or from blast cells restimulated for 5 h with Con A in the presence or absence of CSA. We then assayed lymphokines in conditioned media from cultured *Xenopus laevis* oocytes that had been injected with these RNA preparations.

First, we established that restimulation of T blasts led to the appearance of mRNA for several lymphokines. *Xenopus* oocytes were microinjected with 40 ng of RNA from either Con A-restimulated or unstimulated cultures. After 24–48 h of incubation, the oocyte incubation medium was assayed for lymphokine biological activity (Table V). IL-2,  $\gamma$ -IFN, B cell-stimulating factor(s), and

TABLE III  
Effect of CSA on IL-2 Release from PMA-stimulated EL-4

Stimulation	[ <sup>3</sup> H]TdR incorporated after 7 or 20 h culture of:			
	Cell extracts		Conditioned medium	
	7	20	7	20
None	1,426	1,450	1,817	1,242
EL-4 + PMA	51,050	94,687	65,200	251,228
EL-4 + PMA + CSA	2,291	1,613	1,713	3,073

Conditioned medium (10  $\mu$ l) and cell extracts (20  $\mu$ l) were derived from EL-4 stimulated with PMA (10 ng/ml) in the presence or absence of CSA (1  $\mu$ g/ml) for 7 or 20 h. Data are cpm [<sup>3</sup>H]TdR incorporated in an IL-2 assay.

TABLE IV  
Translation in *Xenopus laevis* Oocytes of EL-4 mRNA: Effect of CSA on IL-2 Activity

Source of mRNA	IL-2 activity ([ <sup>3</sup> H]TdR cpm)
None	1,350
EL-4	1,210
EL-4 + PMA (4 h)	15,070
EL-4 + PMA (7 h)	29,807
EL-4 + PMA + CSA (4 h)	1,870
EL-4 + PMA + CSA (7 h)	1,083

Conditioned medium was derived from groups of oocytes microinjected with 40 ng mRNA from EL-4 cells that were PMA induced (10 ng/ml) or not induced (4 or 7 h), in the presence or absence of CSA (1  $\mu$ g/ml). The equivalent of medium conditioned by 2.5 oocytes was assayed for IL-2.

TABLE V  
Translation in *Xenopus laevis* Oocytes of mRNA from Mitogen-activated T Cell Blasts: Effect of CSA

Lymphokine assayed	Source of mRNA and duration (h) of culture					
	Control		Con A		Con A + CSA	
	24	48	24	48	24	48
IL-2*	1,159	1,254	12,523	4,319	1,290	1,169
Interferon <sup>†</sup>	<4	<4	300	1,000	<4	10
B cell-stimulating factor(s) <sup>‡</sup>	0	0	38	240	0	3
Cytotoxic differentiation factor(s) <sup>§</sup>	1	1.4	9.3	11.2	1.1	1.5

Conditioned medium from groups of oocytes cultured 24 or 48 h, after microinjection of 40 ng mRNA, was assayed. The mRNA was from control blasts or blasts restimulated with Con A with or without CSA for 5 h. The equivalent of medium conditioned by 2.5 oocytes was assayed for lymphokine activity.

\* Results are expressed as [<sup>3</sup>H]TdR cpm incorporated.

† Results are expressed as interferon units.

‡ Results are expressed as PFC for individual cultures.

§ Results are expressed as the percent <sup>51</sup>Cr released in a 4-h lectin-dependent cytotoxic assay.



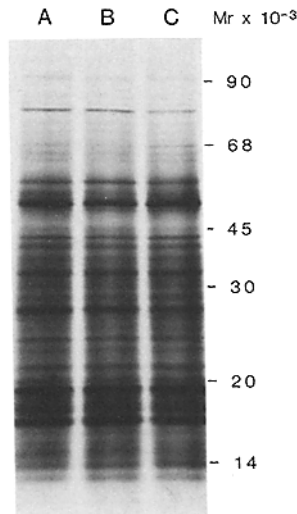


FIGURE 4. Autoradiogram of SDS-PAGE of translation products of activated T cell mRNA. Poly(A)<sup>+</sup> RNA (5  $\mu$ g) extracted from control blast cells (A), Con A-treated blasts (B), or Con A/CSA-treated blasts (C) were translated in a cell-free system. Aliquots of translation products were analyzed in a 6–15% SDS-polyacrylamide gel.

cytotoxic differentiation factor(s) activities were detectable, but only in the medium conditioned by oocytes that had received poly(A)<sup>+</sup> RNA from mitogen-restimulated cultures vs. control blasts.

When mRNA was isolated from T blasts restimulated in the presence of CSA, no detectable lymphokine activity was found in the conditioned medium of oocytes (Table V). Therefore, CSA blocks the induction of active lymphokine mRNA.

To analyze if CSA and Con A would alter the levels of mRNA for bulk cellular proteins, we translated mRNA from control, and Con A- and Con A/CSA-restimulated blasts in a cell-free system. The pattern of synthesized proteins was very similar using each of the three sources of mRNA (Fig. 4). This suggests that the effects of Con A and CSA on lymphokine mRNA are selective.

### Discussion

*Lymphokine Release from T Lymphoblasts.* T lymphoblasts that have been activated for 3 d with the mitogen Con A do not produce or secrete lymphokines constitutively. Instead, lymphokine release requires restimulation with Con A (4). This conclusion applies to a large group of lymphokines, including IL-2,  $\gamma$ -IFN, and B cell- and CTL-stimulating factors (Fig. 1, Table I).

Induction of lymphokine synthesis can be attributed to the appearance of biologically active lymphokine mRNA. Within a few hours of restimulation, IL-2 mRNA appeared and this induction corresponds well with the increase of lymphokine seen in cell extracts and the conditioned medium. The effect was

not limited to IL-2 mRNA, since we succeeded in detecting active mRNAs for  $\gamma$ -IFN as well as B cell- and CTL-stimulating factors (Table V).

If one considers the Con A blast as a model for a sensitized T cell generated during an immune response, then these sensitized cells are exquisitely dependent upon restimulation for the release of lymphokine. It appears that there is no constitutive release or protein stores of these important mediators. Cellular immune responses involve the emigration of large numbers of T blasts into the efferent lymph (21, 22). Presumably the blasts emigrate to inflammatory sites where antigen must be encountered for lymphokine mRNA and protein synthesis to be induced.

*Effects of CSA on Lymphokine Release.* A substantial body of data demonstrates the immunosuppressive properties of the fungal metabolite, CSA. Several T cell responses, such as the mixed leukocyte reaction, lectin mitogenesis, and delayed hypersensitivity, are blocked when the drug is given early in the response (5-9, 23, 24). Our data indicate that CSA inhibits lymphokine release from preformed T blasts by blocking the induction of active lymphokine mRNA. The inhibition applies to a variety of lymphokines (Table V), but not to mRNA's mediating bulk protein synthesis (Fig. 4). Also, CSA did not block several other Con A-stimulated events, such as the overall increase in cell protein synthesis that occurs with Con A, and some changes in the pattern of polypeptides that are induced by lectin (Table II and Fig. 3).

CSA inhibited EL-4 cells similarly to Con A blasts (Tables III and IV). With EL-4, PMA was used to induce active IL-2 mRNA. These data indicate that CSA selectively blocks the expression of lymphokine mRNA in response to several stimuli.

The mechanism by which CSA inhibits lymphokine mRNA is not yet clear. It is possible that CSA directly interferes with the transcription of new lymphokine mRNA, or the drug could alter the function of other products that are essential for the expression of active message. Analysis with cloned probes will resolve this point.

CSA is being used clinically to suppress the immune response to organ transplants. The drug must be given early, possibly to prevent the development of CTL, since CTL function seems not to be sensitive to CSA (Table II). Our data suggest that CSA also may be useful in suppressing T cell-mediated responses that require lymphokine release, including responses by T lymphoblasts. For example, CSA blocks the expression of delayed-type hypersensitivity in antigen-primed animals (23, 24) and this block could be at the level of lymphokine release. Also, tissue damage in diseases like rheumatoid arthritis or sarcoidosis may be mediated by the production of lymphokines by sensitized T cells present in joints or lungs (25, 26). CSA might be an effective immunosuppressive agent during active disease.

### Summary

Three-day, concanavalin A-induced T lymphoblasts have been used as a model to study lymphokine release from sensitized T cells. The blasts responded to interleukin 2 (IL-2) but did not constitutively produce this or other lymphokines. After mitogen restimulation, blast cells synthesized IL-2 as well as  $\gamma$ -interferon,

B cell-stimulating factor(s), and cytolytic differentiation factor(s). This production resulted from the induction of biologically active lymphokine mRNA.

Cyclosporin A (CSA), a potent immunosuppressive agent, strongly inhibited synthesis of IL-2,  $\gamma$ -interferon, and B cell- and CTL-stimulating factor(s), from mitogen-restimulated T blasts. In contrast, CSA did not block the cytolytic activity of the T blasts, nor modify bulk protein synthesis induced by Con A. CSA also blocked lymphokine release from a phorbol myristate acetate-stimulated thymoma cell line, EL-4. The effect of CSA was to block the induction of active lymphokine mRNA, as assayed in an oocyte translation system. This selective inhibition of lymphokine mRNA suggests that CSA may be useful in the therapy of inflammatory, lymphokine-mediated disease states.

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*Note added in proof:* While this article was in press, Krönke et al. (*Proc. Natl. Acad. Sci. USA*, 1984, 81:5214) published results demonstrating that CSA inhibits IL-2 gene expression at the level of mRNA transcription in the human leukemic T cell line Jurkat.

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