

## PERFORIN AND SERINE ESTERASE GENE EXPRESSION IN STIMULATED HUMAN T CELLS

Kinetics, Mitogen Requirements, and Effects of Cyclosporin A

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Several products of CTL and NK cells, including a pore-forming protein (PFP;<sup>1</sup> perforin or cytolyisin) (1-4) and several serine esterases (SE) (5-12), have been cloned and sequenced. These proteins are localized to cytoplasmic granules and represent the known structural components of the pore-formation and granule exocytosis models for cell-mediated killing (13-17). Granule mediators are expressed by CTL and NK cell lines maintained with IL-2, but there is still little information on primary effector lymphocyte populations. Moreover, to date, most of the studies have been performed in the murine system. Here, using cDNA probes encoding two SE (SE 1/granzyme A/Hanukah Factor and SE 2/granzyme B) and the recently cloned perforin cDNA, we have initiated studies aimed at understanding how the production of these proteins is regulated in human primary T lymphocytes. We have examined RNA accumulation in response to a number of well-known T cell mitogens and have determined the effect of cyclosporin A (CsA) on this inducible response.

### Materials and Methods

*Materials.* Lectins Con A and PHA, and PMA, were purchased from Sigma Chemical Co. (St. Louis, MO). Restriction endonucleases were obtained from United States Biochemical Corp. (Cleveland, OH). CsA and an immunologically inactive analogue cyclosporin H (CsH) were obtained from Sandoz Co. (Basel, Switzerland). Human rIL-2 was generously provided by Biogen (Cambridge, MA) and Cetus Corp. (Emeryville, CA).

cDNA probes were obtained as follows. Granzyme B (a 0.9-kb Eco RI segment) and actin (an Eco RI-Xho I segment) were obtained as described (8); granzyme A (a 0.9-kb Eco RI-Bam HI segment) was generously provided by Dr. I. L. Weissman of Stanford University, Palo

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<sup>1</sup> *Abbreviations used in this paper:* CsA, cyclosporin A; CsH, cyclosporin H; LT, lymphotoxin; PFP, pore-forming protein; SE, serine esterase.

Alto, CA (11); human TNF- $\alpha$ /cachectin (a 300-b Pvu II fragment) and lymphotoxin (LT, or TNF- $\beta$ ) (a 950-b Eco RI segment) were obtained from Dr. Michael Sheppard, Genentech Inc., South San Francisco, CA (18).

**Cell Lines.** Human T cell lines MOLT-4, CEM, HPB-ALL, HUT 78, and RPMI-8402, erythroleukemia cell line K562, promyelocytic leukemia cell line HL-60, histiocytic leukemia cell line U937, B lymphoma cell line NALM-16, and Burkitt lymphoma cell line Raji were obtained from the American Type Culture Collection (Rockville, MD). The B lymphoblastoid cell lines, COX, DBB, IM-9, and MANN have been characterized and described elsewhere (19, 20). SK007 is a human plasma cell line.

**PBL.** Human PBMC were obtained from buffy coats of normal donors (New York Blood Center, New York) by Ficoll-Hypaque gradient centrifugation. To enrich for T lymphocytes, PBMC were subjected to plastic adherence and passage over nylon wool columns to deplete monocytes and B lymphocytes. Monocyte-depleted PBL or nylon wool-enriched T lymphocytes were each cultured at  $5 \times 10^6$  cells/ml in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 5% FCS (Gibco Laboratories), and different mitogens: PMA at 10 ng/ml, Con A at 5  $\mu$ g/ml, PHA at 20  $\mu$ g/ml, or rIL-2 at 100 U/ml. At the indicated times, cells were washed and subjected to RNA extraction. T lymphoblasts were prepared as described in detail elsewhere (21). Briefly, PBMC were cultured at  $2 \times 10^6$  cells/ml in medium containing 2  $\mu$ g/ml of PHA for 4–5 d. The T blasts were collected, washed, and cultured in medium in the presence or absence of stimulants as described above.

Subpopulations of T lymphocytes were further fractionated by negative selection using a panning technique. Cells were incubated with the mAbs that recognize cell surface molecules CD4 (OKT4), CD8 (OKT8), or CD11b complement receptor (OKM1), respectively, for 1 h on ice. Cell supernatants of hybridomas OKT4, OKT8, and OKM1 hybridoma (American Type Culture Collection) were used at a 1:3 final dilution. Cells were washed three times and the antibody-coated cells were centrifuged onto plastic dishes coated with goat anti-mouse Ig (Organon Teknika-Cappel, Malvern, PA). Nonadherent cells were removed and reapplied to new coated dishes two more times. After the third round of panning, cells were cultured with various mitogens for the indicated times before RNA extraction. Homogeneity of cell populations obtained by this panning procedure was ascertained by cytofluorographic analysis (FACS), which revealed undetectable levels of the phenotypic markers against which cells had been negatively selected in the panning procedure. Plastic-adherent cells were used as accessory cells at the ratio of 1:5 (one accessory cell equals five T cells).

Blood samples from a patient having marked elevation in peripheral blood large granular lymphocytes were obtained from Dr. M. Andreeff (Memorial Hospital for Cancer and Allied Diseases, New York). 80% of PBMC from this patient were phenotypically NK cells (Leu-11<sup>+</sup>, Leu-19<sup>+</sup>), as judged by cytofluorographic analysis. After culture of these PBMC in medium containing rIL-2 at 50 U/ml, >98% of these cells were of the NK phenotype. These cells were used in Fig. 1 (last two lanes). Allogeneically stimulated bulk lymphocyte populations were derived from an MLC using PBL from two HLA-unmatched healthy donors. The MLC-derived cells were periodically restimulated with the corresponding allogeneic cells and expanded in IL-2-containing medium as above (material used for Fig. 1, first lane).

**cDNA Clone for Human Perforin.** A cDNA library of MLC-derived human lymphocytes was constructed in bacteriophage  $\lambda$ gt11, as described elsewhere (8). The library was screened by plaque hybridization using a 33-base oligonucleotide probe, CTTGCACTCT-GAGCGTGCGGCTGTGTGGCACGG (5' to 3'), which is complementary to nucleotides 63–96 of a published cDNA sequence of human perforin (2). The clones that hybridized with this probe were plaque purified and subcloned into the plasmid vector pUC18. A clone (designated HP-10) with a 2.5-kb insert encoding the full-length human perforin was used in all subsequent experiments. Plasmid DNA containing HP-10 was sequenced by the dideoxynucleotide chain termination method (22).

**Southern (DNA) Blot Analysis.** Genomic human DNA was prepared and digested with various restriction enzymes. 10  $\mu$ g of digested DNA was fractionated through a 0.7% agarose gel and capillary transferred to a nylon membrane (Micron Separations Inc.). Prehybridization and hybridization were conducted at 42°C in 50% formamide, 5 $\times$  Denhardt's (1 $\times$  Denhardt's: 0.02% BSA, 0.02% Ficoll, 0.02% polyvinylpyrrolidone), 5 $\times$  SSC (1 $\times$  SSC: 0.015

M Na Citrate, 0.15 M NaCl, pH 7.0), 1% SDS, and 100  $\mu\text{g}/\text{ml}$  of salmon sperm DNA, for 3 and 16 h, respectively. The cDNA probe was nick-translated using  $\alpha$ -[ $^{32}\text{P}$ ]dCTP (New England Nuclear, Boston, MA). After hybridization, the blot was washed twice with  $2\times$  SSC/0.1% SDS at room temperature for 15 min, followed by two 15-min washes with  $0.5\times$  SSC/0.1% SDS at  $60^\circ\text{C}$ . The autoradiogram was exposed at  $-70^\circ\text{C}$  for 3 d with intensifying screens.

**Northern (RNA) Blot Analysis.** 10  $\mu\text{g}$  of total cellular RNA or 1  $\mu\text{g}$  of poly(A)<sup>+</sup> RNA was isolated using a modified guanidinium isothiocyanate/CsCl method (23), fractionated on 1% agarose-formaldehyde gels, transferred to GeneScreen Plus membrane (New England Nuclear), and probed with  $^{32}\text{P}$ -labeled probes. The blots after hybridization were washed two times with  $2\times$  SSC/1% SDS at room temperature for 15 min, followed by two washes with  $0.5\times$  SSC/1% SDS at  $60^\circ\text{C}$ . Autoradiography was performed with intensifying screens for 12 h to 5 d. In some experiments, the signal intensities obtained with perforin, SE 1, and SE 2 were normalized against the intensity seen with actin cDNA probe.

## Results

**Isolation of a Human Perforin cDNA Clone.** A cDNA library was constructed from human T lymphocytes stimulated in the MLC, and the library was screened with an oligonucleotide probe complementary to a published sequence of human perforin (2). Of five positive clones, one, designated HP-10, was sequenced. HP-10 contains a 2.5-kb insert that includes a poly(A) stretch preceded by the consensus polyadenylation signal AATAAA. 700 bases of HP-10 were sequenced and were shown to be identical to those published elsewhere (reference 2; data not shown). The sequence information indicates that HP-10 encodes for full-length human perforin with extension of noncoding region in both 5' and 3' ends. Southern blot analysis of human genomic DNA with HP-10 showed that human perforin is encoded in a single locus in the human haploid genome (not shown). Perforin coding sequences were contained within 10 kb of genomic DNA.

To verify the cellular distribution of the cloned perforin transcript, Northern blot analysis was conducted on activated lymphocyte populations and on a panel of human cell lines (Fig. 1). HP-10 hybridized with a unique 2.9-kb mRNA species from allogenically stimulated, IL-2-expanded T lymphocytes (Fig. 1, first lane) and NK cells obtained from a leukemic patient (Fig. 1, last two lanes), which effectively kill appropriate allogenic target cells and K562 cells, respectively. Perforin mRNA levels increased in NK leukemia cells after culture with rIL-2 for 20 d (Fig. 1, compare the last two lanes). These same cell populations contained mRNAs for SE 1, SE 2, and actin (Fig. 1). Actin was used as an internal control for all the Northern blots since minimal changes in actin RNA were observed in response to cell stimulation with mitogens. After short-term treatment of NK leukemia cells with rIL-2, the mRNA level for SE 1 was more pronouncedly increased (5.6-fold when compared with non-stimulated NK leukemia cells) than that of SE 2 (2.7-fold). Of the 16 noncytotoxic human cell lines tested, only HUT-78, an HTLV-1-transformed T lymphoma cell line, produced low levels of perforin mRNA, and none produced detectable levels of SE 1 or SE 2 mRNAs.

**Induction of Perforin and SE in Primary Lymphocyte Populations and Effects of CsA.** PBL from healthy donors were stimulated with several mitogens single or in concert. As shown in Fig. 2, resident T cells expressed little or no mRNAs specific for perforin, SE 1, or SE 2. The addition of rIL-2 at 100 U/ml (Fig. 2 A), or a combination of lectin (Con A or PHA) and PMA (Fig. 2 B), induced all three mRNAs. mRNA

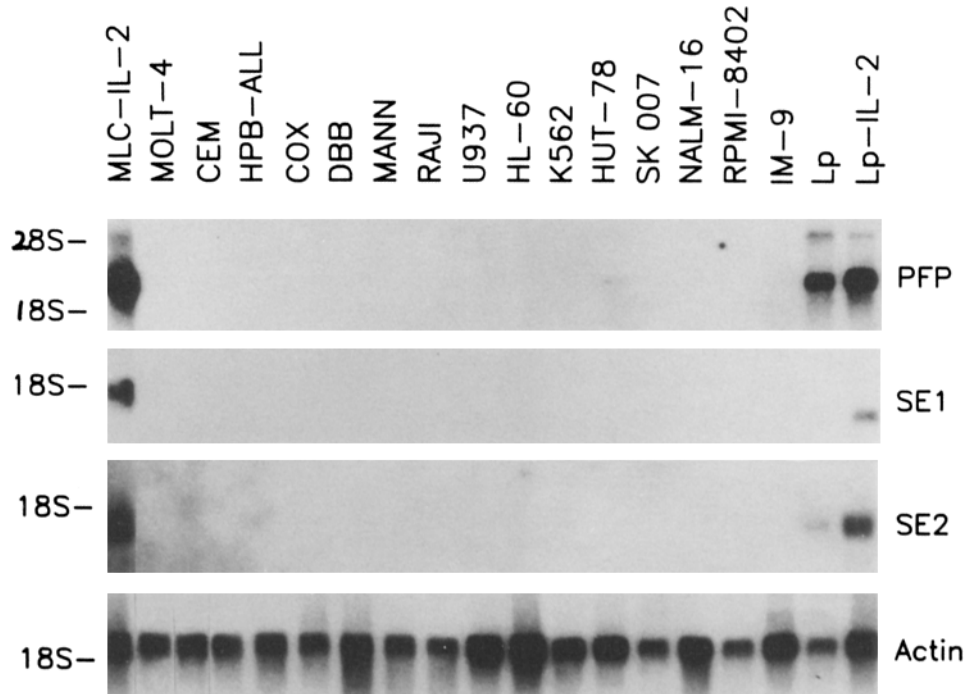
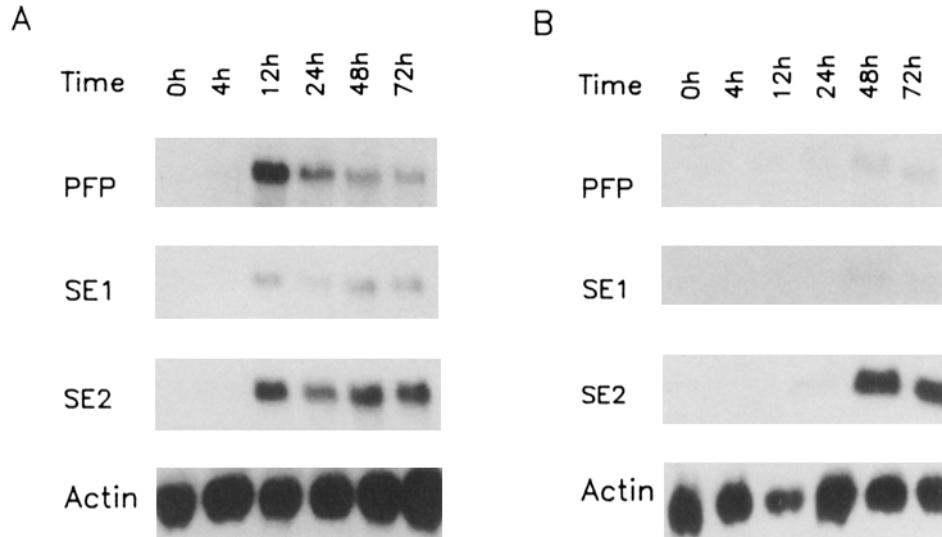


FIGURE 1. RNA blot analysis to verify cell-type distribution of human perforin and SE. Lanes contain poly(A)<sup>+</sup> RNA (1  $\mu$ g per lane) and were hybridized with probes specific for PFP/perforin, SE 1, SE 2, and actin. The same blot was rehybridized with several cDNA probes. MLC-IL-2 represents a bulk (i.e., uncloned) culture of allogeneically stimulated, IL-2-expanded human PBMC. Lp represents the cells of a patient with a marked leukocytosis involving NK cells (Materials and Methods). Lp-IL-2 RNA was derived from the same cells cultured with rIL-2 for 20 d. Autoradiography was performed for 3 d.

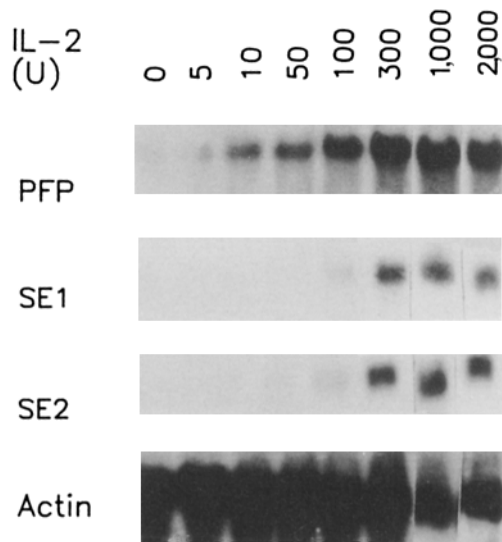
induction by either rIL-2 or a combination of PMA and lectin followed different time courses (compare Fig. 2, *A* and *B*). Perforin and the two SE were quickly induced by rIL-2 (100 U/ml): low levels of mRNAs were detected 4 h after stimulation, peak mRNA levels occurred at 12–24 h, and thereafter, the mRNA levels decreased. In contrast, the combination of PMA and lectin caused a slower accumulation of mRNA levels for all three transcripts, taking 12–24 h for mRNAs to be detected and 48–72 h to peak. The extent of mRNA accumulation for all three transcripts was augmented with increasing doses of rIL-2, as shown in Fig. 3. For perforin, as little as 10 U/ml of rIL-2 resulted in increased mRNA levels after 12 h of treatment, whereas induction of SE 1 and SE 2 mRNAs required higher doses of rIL-2. For the two SE, we observed that, in general, SE 1 levels were upgraded more readily by IL-2, while SE 2 appeared to respond better to a combination of lectin plus PMA; however, this conclusion could not be generalized, as PBL from individual donors showed marked variability (see Fig. 2 *B* for example).

Lectin-primed T cells (T blasts) had previously been shown to be a useful source for studies of the control of several T cell mRNAs (21). We first verified whether the requirements for induction of all three transcripts studied here were similar to



**FIGURE 2.** Induction of perforin and SE mRNAs in PBL. Human PBL derived from buffy coats of normal donors were stimulated with 100 U/ml of rIL-2 (A) or 10 µg/ml of Con A and 10 ng/ml of PMA (B) for the indicated periods of time. 10 µg of total cellular RNA in each lane was resolved on the agarose gel and analyzed sequentially with cDNA probes specific for PFP/perforin, SE 1, SE 2, and actin. The autoradiograms in A were exposed for 1 d. The autoradiograms in B were exposed for 7 d, with the exception, however, of the blot hybridized with actin cDNA, which was exposed for 1 d.

those established for unprimed populations (Fig. 4). Perforin mRNA was detected in most T blast populations examined. IL-2 alone, or a combination of lectin and PMA, further increased perforin mRNA levels. SE 1 and SE 2 mRNAs were similarly upregulated. In the experiment shown in Fig. 4, SE 1 responded well to IL-2



**FIGURE 3.** Dose-dependent induction of human perforin and SE mRNAs by rIL-2. Human peripheral blood T lymphocytes were cultured for 12 h in the presence of the indicated amounts of rIL-2. Total cellular RNA (10 µg per lane) was extracted and analyzed by RNA blot analysis using cDNA probes for the indicated transcripts. The autoradiogram was exposed for 24 h.

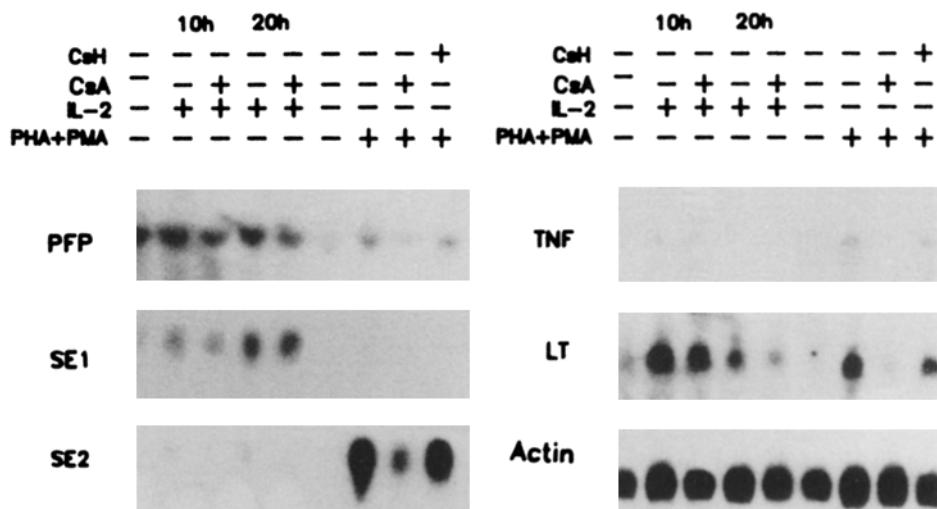


FIGURE 4. RNA blot analysis of expression of human perforin, SE, TNF, and LT mRNAs in T lymphoblasts. PHA-primed T lymphoblasts were prepared as described in Materials and Methods. The blasts were stimulated for 10 or 20 h with rIL-2 (100 U/ml) and 16 h with PHA (10  $\mu$ g/ml) plus PMA (10 ng/ml). In the same experiments, some T lymphoblasts were stimulated, as described above, in the presence of CsA or CsH (both at 1  $\mu$ g/ml). Total cellular RNA was extracted and analyzed with various cDNA probes. Each lane contains 10  $\mu$ g/ml of RNA. The autoradiograms were exposed for 3 d (PFP, SE 1, SE 2, LT, and TNF) or for 8 h (actin).

but not to PHA/PMA stimulation, and conversely, SE 2 responded well to PHA/PMA treatment but only poorly to IL-2 stimulation. We also monitored the levels of two cytokines: TNF and LT (Fig. 4). TNF mRNA increased weakly in response to PHA/PMA treatment, whereas LT mRNA was augmented in response to both IL-2 and PHA/PMA. For all five mRNAs, addition of 1  $\mu$ g/ml of CsA, but not the nonimmunosuppressive analogue CsH, partially abrogated the stimulatory effect of IL-2 and PHA/PMA (Fig. 4 and Table I). The induction requirements for perforin and

TABLE I  
*Inhibitory Effect of CsA on Expression of Perforin and SE  
in Stimulated T Lymphoblasts*

mRNAs	Percent inhibition of mRNA stimulated with:	
	IL-2	PHA/PMA
Perforin	42	74
SE 1	14	ND
SE 2	60	65
TNF	ND	79
LT	27	81

The autoradiograms shown in Fig. 5 were scanned with a video densitometer. The IL-2-induced signals were taken from the 10-h time point. The signals for the indicated transcripts were normalized against the signal for actin. For determination of percent inhibition, the signals given by treated cells were compared with those produced by untreated cells at time 0. The percent inhibition could not be determined where indicated because signals were too weak.

SE mRNAs in T blasts were in general similar to that observed with resident T cells (data not shown).

We then compared the response of cells to PMA plus single stimuli with that to the mAb OKT3, which specifically ligates the TCR-CD3 complex. For T blasts, PMA alone caused marked induction of SE 2 mRNA but had no effect on perforin and SE 1 mRNAs (Fig. 5). Lectin alone induced only the perforin mRNA to an appreciable degree (twofold; data shown only for Con A in Fig. 5; similar observations were observed with PHA, data not shown). On the other hand, the combination of PMA and lectin had a synergistic effect on both perforin and SE 2 mRNAs; in most cases SE 2 mRNA was induced strongly by this treatment (190-fold in the experiment shown in Fig. 5). SE 1 mRNA responded poorly to PMA plus lectin (in Fig. 5, there was no increase at all).

The mitogenic OKT3 mAb had either no or very little independent effect on any of the mRNAs we studied when used alone, but was markedly synergistic with PMA or adherent accessory cells (see Materials and Methods) in inducing perforin, SE

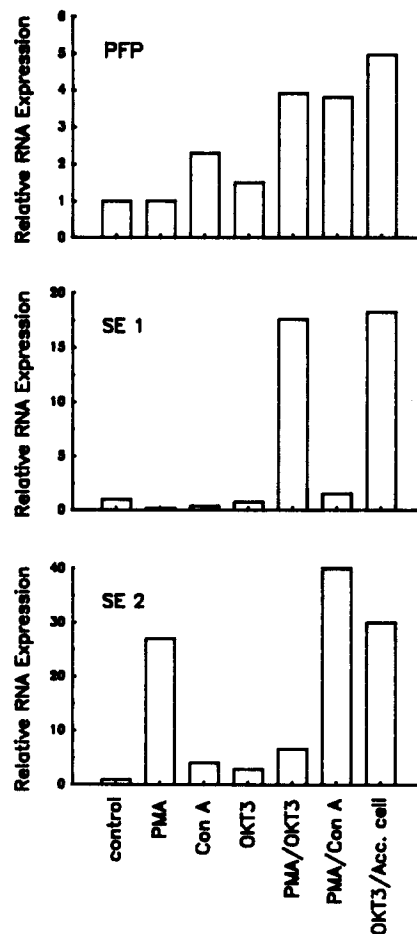


FIGURE 5. Effect of mitogens on mRNA levels of perforin and SE in T lymphoblasts. PHA-primed T lymphoblasts were stimulated for 24 h with the indicated reagents before RNA blot analysis. PMA was used at 10 ng/ml and Con A at 10  $\mu$ g/ml. OKT3 and adherent accessory cells were used as described in Materials and Methods. Densitometric scanning was performed on the hybridization signals obtained from the RNA blots. The signals for perforin, SE 1, and SE 2 were normalized against that for actin. The signal ratios obtained for time 0 were assigned to equal unity. For SE 2, the signal for PMA/Con A was 190-fold over control.

1, and SE 2 mRNAs (Fig. 5). Essentially similar results were obtained with unprimed T cells (data not shown).

A dose-response experiment was performed to establish the effective dose of CsA in blocking induction of the three specific mRNAs studied here. For T blasts, as little as 30 ng/ml of CsA, added in the presence of PMA and Con A, produced a partial inhibitory effect on the induction of all three mRNAs (Fig. 6). CsA partially inhibited the mRNAs increase mediated by PMA/Con A. At 0.9  $\mu\text{g/ml}$ , for example, CsA blocked 20–59% (range for the three mRNAs) of the induction response. Similar inhibitory effects of CsA were observed with unprimed T cells (not shown). In the case of rIL-2, a partial inhibitory effect of CsA for all three mRNAs could be observed at 12, 24, and 48 h after stimulation (Fig. 7; data shown only for resting PBL). Thus, at 48 h of stimulation with IL-2, for example, the mRNA increase for perforin, SE 1, and SE 2 was inhibited 43, 55, and 55%, respectively, by CsA.

*mRNA Levels in T4 and T8 Subsets.* Highly enriched populations of T8<sup>-</sup>, T4<sup>-</sup>, and OKM1<sup>-</sup>T4<sup>-</sup> cells were prepared by negative selection using a panning technique and were stimulated with various mitogens (Fig. 8; data shown only for IL-2). Panning with OKM1 removes precursors of NK and/or lymphokine activated killer (LAK) cells that are capable of killing K562 targets (J. W. Young, manuscript in preparation). OKM1<sup>-</sup>T4<sup>-</sup>-depleted T8<sup>+</sup> cells accounted for most of the mRNA increase induced by IL-2 (Fig. 8). The signal associated with OKM1<sup>-</sup>T4<sup>-</sup> cells could not be attributed to contaminating NK cells since undetectable levels of OKM1<sup>+</sup> cells were observed in the final preparations. Maximally stimulated T8<sup>-</sup> (mainly T4<sup>+</sup>) cells also expressed a small amount of perforin and SE (particularly SE 1) mRNAs. In preliminary experiments, T8<sup>-</sup>OKM1<sup>-</sup> cells (>99% T4<sup>+</sup>) also presented measurable levels of perforin and SE mRNAs upon stimulation with IL-2 (not shown). Similar results were observed when negatively selected T cells of T4 or T8 pheno-

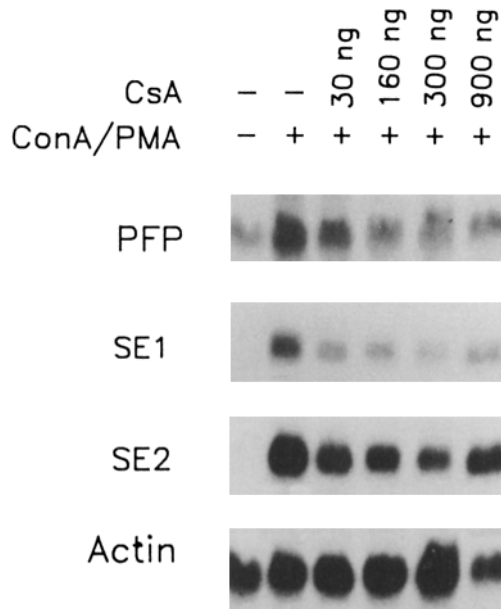


FIGURE 6. Dose dependence of the inhibitory effect of CsA on induction of perforin and SE mRNAs in PHA-primed T lymphoblasts. T lymphoblasts were stimulated with Con A (10  $\mu\text{g/ml}$ ) and PMA (10 ng/ml) for 24 h in the absence or presence of CsA at the indicated concentrations. Total cellular RNA (10  $\mu\text{g}$  per lane) was analyzed with the cDNA probes specific for PFP/perforin, SE 1, SE 2, and actin. The autoradiograms were exposed for 24 h.



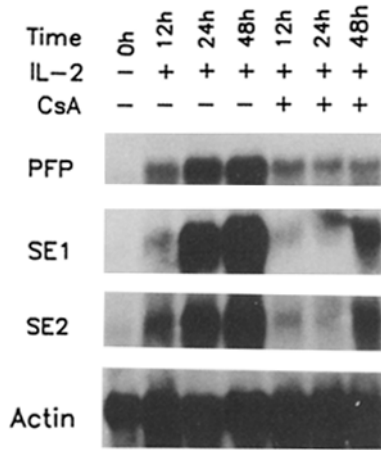


FIGURE 7. Inhibitory effect of CsA on induction of perforin and SE mRNAs in resting T lymphocytes. Human monocyte-depleted PBL were stimulated with rIL-2 (100 U/ml) for 12, 24, and 48 h in the absence or presence of CsA at 1  $\mu$ g/ml. The autoradiograms were exposed for 3 d.

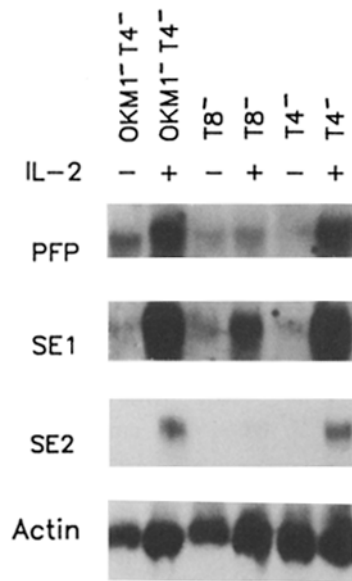


FIGURE 8. Differential expression of perforin and SE transcripts in various T cell subsets. Human peripheral blood T lymphocytes were separated into T4<sup>-</sup>, T8<sup>-</sup>, T4<sup>-</sup>OKM1<sup>-</sup> populations by negative selection using a panning technique. The cells were cultured with or without rIL-2 (100 U/ml) for 24 h before being processed for RNA blot analysis. The autoradiograms were exposed for 3 d.

types were stimulated with PMA/lectin (data not shown). That is, induction of all three mRNAs occurred mainly in T cells of the T8 phenotype, while T8<sup>-</sup>T4<sup>+</sup> cells produced low but measurable levels of those same mRNAs.

### Discussion

The role of perforin and SE in mediating cytotoxicity has not been settled, in part because the distribution of these mediators in primary effector lymphocyte populations is virtually unknown. The lack of knowledge on these mediators is more evident in the human system, since there is only a limited amount of biochemical information on human perforin (2, 24, 25) and SE (26-28), when compared with the much more detailed analyses with murine cells (reviewed in references 13-16). Human peripheral blood T cells represent an attractive model for studying granule medi-

ators because large numbers of cells can easily be obtained and because much is known about the induction of a number of lymphokine and other growth-related mRNAs after polyclonal stimulation. Using cDNA probes specific for human perforin and two SE mRNAs, we studied their modulation in unprimed and primed T cells in response to several T cell mitogens. We found that the stimulation of resting T cells, or the restimulation of lymphoblasts, induces the formation of these specific mRNAs. Four parameters were monitored: stimuli, lymphocyte subsets, kinetics, and effect of CsA.

Unprimed T cells in general did not produce measurable levels of these mRNAs. However, in a small number of unprimed T cell preparations used in our study, we were able to detect low levels of perforin and SE mRNAs. Optimal mRNA induction required the concerted action of PMA and one of three other stimuli used: lectin, OKT3 mAb, or accessory cells. There was little and sometimes no increase in perforin and SE (1 and 2) mRNAs when the T cells were triggered independently by lectin, PMA, OKT3, or accessory cells. The requirement for PMA plus a mitogen (OKT3 or lectin) for optimal polyclonal activation is consistent with previously published results that suggest the need for two or more signals for resting lymphocytes to become active and proliferation competent (see reference 29 for review). We noticed that accessory cells could substitute effectively for PMA when used in combination with lectin or OKT3. These findings are similar to the stimulation requirements for lymphokine gene expression in T cells (30).

IL-2 alone, on the other hand, markedly induced the expression of perforin and SE 1, and, to a lesser degree, SE 2. NK and LAK cells are known to respond directly to IL-2 with enhanced lymphokine production, cytotoxicity, and proliferation (31). However, our results show that resting, unprimed T cells depleted of NK/LAK precursors using the OKM1 mAb responded promptly to IL-2 with enhanced perforin, SE 1, and SE 2 mRNA accumulation.

Among T cells, the T8 (CD8) subset appeared to be mainly responsible for perforin and SE production. However, we have consistently detected perforin and SE mRNAs in the T4 (CD4) subset in different experiments (data shown here and unpublished observations). Since perforin was also detected in a cloned CD4<sup>+</sup> lymphocyte cell line (HUT-78; Fig. 1), it is possible that perforin may indeed be produced by a subpopulation of CD4<sup>+</sup>, class II MHC-restricted CTL (32, 33).

With regard to kinetics, the induction of perforin, SE 1, and SE 2 mRNAs in both primed and unprimed T cells appeared earlier with the IL-2 treatment than with the treatment with PMA plus lectin, OKT3 mAb, or accessory cells. IL-2 was a more effective inducer than PMA/lectin. The early mRNA induction effect produced by IL-2 is consistent with the time course of induction observed with other lymphokine mRNAs (34-37). This induction effect is probably not mediated through the IL-2-R chain P55, since this chain is not expressed on resting T cells (38); and in our studies, the p55 mRNA appeared much later than the perforin/SE signals (S. Rafii, unpublished observations).

The onset of the PMA response involves likely activation of protein kinase C, which has been suggested before to play a direct role in T cell activation (39, 40) and in CTL function (41).

The profound immunosuppressive effects of CsA on T cells are well known and

appear to be explained in part by its inhibitory activity on lymphokine gene transcription (21, 30, 42-46). CsA is known to selectively inhibit T cell activation mediated by some but not all mitogens (46, 47). We obtained only a partial abrogation of mRNA levels in T cells that have been challenged with IL-2 or PMA/lectin in the presence of CsA. It should be noted that we consistently observed an inhibitory effect of CsA on IL-2-mediated induction of perforin and SE mRNAs in both primed and unprimed T cell populations. This is a surprising finding, since CsA is largely ineffective on IL-2-mediated induction of IFN- $\gamma$  and IL-2-R mRNAs (reviewed in reference 46).

The findings presented here do not address the question of whether perforin and SE are directly involved in cell-mediated killing. However, since perforin and SE mRNAs are increased by mitogenic signals known to induce cytotoxic function, it appears that these transcripts are modulated in T cells in a fashion appropriate for a killing role. Experiments are presently being designed to further analyze the expression and control of these genes in stimulated T cells.

### Summary

A pore-forming protein (PFP; perforin) and various serine esterases (SE) have been identified in the cytoplasmic granules of CTL and NK cells. Perforin and several SE have recently been cloned. Northern blotting analysis was performed here using cDNA probes specific for human perforin and two SE (SE 1/HS and SE 2/GB) to monitor the levels of specific mRNAs in mitogen-stimulated primary human T cells. These mRNAs were rapidly induced by IL-2 with optimal responses at 300 U/ml. After IL-2 treatment, mRNAs for perforin, SE 1, and SE 2 peaked at 12-24 h and decreased after 48 h. The three mRNAs were also induced in T cells treated with a combination of PMA plus lectin, OKT3 mAb, or plastic-adherent accessory cells. However, the induction induced by PMA/mitogen followed a slower kinetics, peaking at 48 h. In general, we found that SE 1 mRNA was more readily induced by IL-2, while SE 2 responded better to PMA/mitogen. Similar patterns of mRNA expression were observed for both unprimed T cells and PHA-primed T blasts. After stimulation with IL-2 and PMA/mitogen, the T8<sup>+</sup> subset was shown to be the main producer of perforin, SE 1, and SE 2. Low levels of all three mRNAs, however, were also detected in the T4<sup>+</sup> subset. The induction of all three mRNAs by either IL-2 or PMA/mitogen was partially blocked by the immunosuppressive drug cyclosporin A (CsA), but not by the biologically inactive analogue cyclosporin H. Together, these results point to some similarities and differences with upregulation of granule mediator mRNAs relative to lymphokine mRNAs. Both sets of genes require two signals for their induction by mitogens. In contrast to lymphokines, there is a strong response of granule mRNAs to IL-2, and the induction of these transcripts is only partially blocked by CsA.

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