10. Males were allowed to mount each female ten times and the lordosis quotient (number of lordotic responses/number of mounts × 100) was calculated. Estradiol benzoate (2.5 μg, subcutaneously; Sigma) was administered each day for 2 days before testing. All groups were tested at least once per week. The seven females who received raphe tissue and were examined immunocytochemically also received progesterone and an additional behavior test before being killed. Lordosis scores were maximal (87 ± 13), indicating that the transplants had not interfered with brain centers responsible for lordotic responding.
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Induction of Interleukin 2 Messenger RNA Inhibited by Cyclosporin A

Abstract. *Cyclosporin A blocked production of the lymphokine interleukin 2 by activated T lymphocytes. In a human and a murine cell line this inhibition reflected an absence of interleukin 2 messenger RNA. Under conditions in which these cells are normally stimulated to secrete high levels of interleukin 2, they failed to do so in the presence of cyclosporin A. In both cell lines this failure was accompanied by an absence of interleukin 2 messenger accumulation.*

Cyclosporin A (CsA) is an effective immunosuppressant that has proved useful in organ transplantation. Its mode of suppression may be based, at least in part, on its ability to inhibit production of the lymphokine interleukin 2 (IL-2) by T lymphocytes (*1, 2*). Stimulation of T-lymphocyte growth by IL-2, on the other hand, is not sensitive to CsA (*2–4*). Failure to detect IL-2 synthesis in leukocyte populations in the presence of CsA, however, is not definitive evidence that IL-2 is not being generated. Rapid utilization or destruction of the secreted lymphokine could account for its absence. Furthermore, even if it can be shown that IL-2 is not being produced, it is desirable to determine whether the block created by CsA is at the level of transcription, translation, or post-translational processing. We therefore examined the effects of CsA at the level of IL-2 messenger RNA (mRNA). Several T-lymphocyte lines produce IL-2 in response to stimulation, including mouse T-lymphoma line EL4 (*5*) and human T-cell leukemia line Jurkat (*6*). We found that lines EL4 and Jurkat are both inhibited from producing IL-2 by CsA and that this inhibition is correlated with an absence of IL-2 mRNA measured biologically or chemically.

Subclone EL4.E1 of line EL4 (*5*) was selected for its high level of IL-2 secretion on stimulation with phorbol myristate acetate (PMA). Subclone JEH.3B of the Jurkat line generates somewhat lower levels of IL-2 on stimulation with PMA plus concanavalin A (Con A) than EL4.E1 does. To determine whether synthesis of IL-2 by EL4.E1 and JEH.3B cells is sensitive to CsA, we stimulated both lines in the presence of various concentrations of CsA. CsA blocked secretion of IL-2 by both cell lines (Fig. 1). The concentration of CsA required for 50 percent inhibition, 5 to 10 ng/ml, was similar to concentrations previously found to be effective (*2*). Nearly complete inhibition was observed at 50 ng/ml, and this concentration was used in subsequent experiments. As others have found (*1, 2*), CsA had no effect on DNA synthesis in the IL-2–dependent mouse T-cell line used for assaying IL-2. Furthermore, CsA had no apparent effects on the growth or morphology of line EL4.E1 or JEH.3B.

To determine whether the lack of IL-2 secretion in the presence of CsA was due to a lack of mRNA coding for this lymphokine, we isolated mRNA from cells and translated it in *Xenopus laevis* oocytes (*7, 9*). The biologically active translation products were assayed on the cloned murine cytotoxic T-lymphocyte line MTL2.8.2 (*8*). Stimulation of EL4.E1 cells with PMA induced generation of IL-2 mRNA, as detected by translation in oocytes (Table 1). When CsA was also present in the cell cultures at 50 ng/ml, PMA did not lead to accumulation of translatable IL-2 mRNA. As a control, mRNA was prepared from cells stimulated in the presence or absence of CsA and mixed at harvest. The mixed mRNA preparation gave the expected yield of translated IL-2, demonstrating that the lack of mRNA in the CsA-treated cells was not due to inhibitory material (Table 1). These results indicate that CsA reduced the level of IL-2 mRNA by a factor of at least 10.

To confirm that lack of translation was not due to a peculiarity of the oocyte system, we also translated some mRNA samples in the cell-free wheat germ extract system. This system is much less efficient than microinjected oocytes, but the result was the same, namely that no biologically active IL-2 mRNA could be isolated from EL4.E1 cells stimulated in the presence of CsA.

Similar results were obtained with line

Table 1. Effect of CsA on the level of translatable IL-2 mRNA in EL4.E1 cells. Subclone EL4.E1 of the IL-2–producing T-lymphoma line EL4 (*5*) was grown in RHFM (*7*) supplemented with 10 percent fetal calf serum (FCS). Cells in logarithmic-phase growth were collected and suspended at a density of 1×10^6 per milliliter in RHFM supplemented by 2 percent FCS. A 1.5-liter culture was treated with PMA (group A), a second 1.5-liter culture was treated with PMA plus CsA (group B), and a 1-liter culture was incubated without stimulation (group C). After 16 hours the cells were harvested and 3.5×10^8 cells from each of groups A and B were mixed to form group D, which served as a control to show that CsA-treated samples do not inactivate IL-2 mRNA or prevent its translation. Groups A, B, and D each contained about 7×10^8 cells (PMA stops proliferation, even in the presence of CsA). Group C, which had continued to proliferate, contained 1.8×10^9 cells. Cells were homogenized in 7.4*M* guanidine HCl (*11*), and polyadenylated RNA was isolated as described earlier (*9*). The mRNA was injected (50 nl at 2 mg/ml, 20 oocytes per group) into *Xenopus laevis* oocytes, and these were incubated at 25°C for 63 hours. Media from the groups were pooled and the oocytes were pooled and homogenized. Supernatants and homogenates were assayed on the mouse cytotoxic T-lymphocyte line MTL2.8.2 at various dilutions in triplicate (see legend to Fig. 1). Data from the activity-dilution curves were converted to 30 percent effective dose (ED_{30}) units per milliliter. One ED_{30} unit stimulates 30 percent of the maximum proliferative response under the assay conditions.

Group	Addition*	IL-2 synthesized by oocytes (mean ED_{30} units per milliliter ± standard deviation)
A	PMA (20 ng/ml)	128 ± 7
B	PMA (20 ng/ml) plus CsA (50 ng/ml)	1.4 ± 0.2
C	Medium only	0.4 ± 0.3
D	Cells from groups A and B	60.0 ± 1.1

*Addition to culture of EL4.E1 cells from which polyadenylated RNA was extracted.

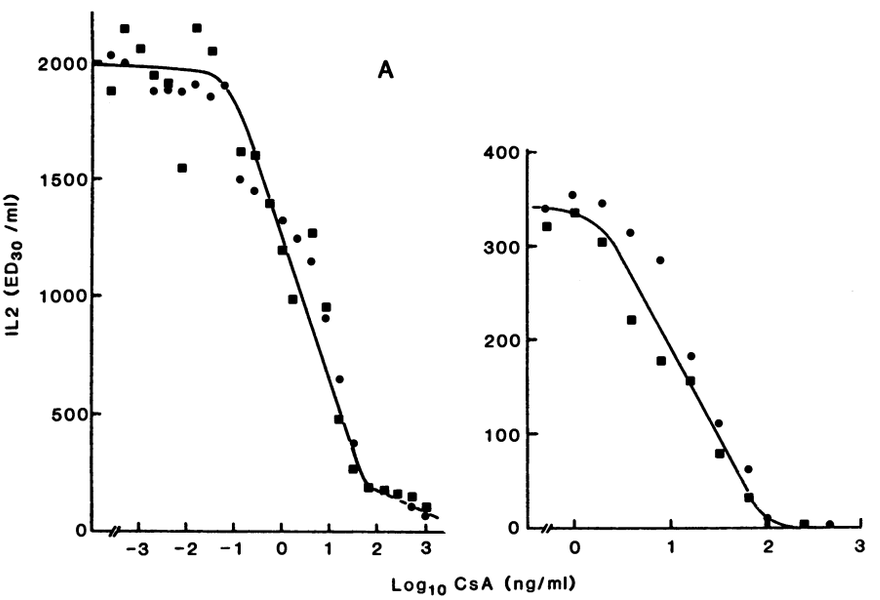
Table 2. Effect of CsA on the level of translatable IL-2 mRNA in human Jurkat cells. Human T-cell leukemia line JEH.3B was grown in RHFM supplemented with 10 percent FCS, collected, and divided into three 1-liter cultures (1×10^6 cells per milliliter). Cultures were incubated with the additions indicated for 16 hours. Approximately 7.0×10^8 viable cells were recovered from groups A and B; 1.3×10^9 cells were recovered from group C. Polyadenylated RNA was extracted from each group of cells and translated in oocytes as described in the legend to Table 1. Oocyte supernatants and homogenates were assayed for IL-2 independently and the total IL-2 content was calculated.

Group	Addition*	IL-2 synthesized by oocytes (mean ED_{30} units per milliliter ± standard deviation)
A	PMA (20 ng/ml) plus Con A (33 μg/ml)	5.49 ± 0.09
B	PMA (20 ng/ml) plus Con A (33 μg/ml) plus CsA (50 ng/ml)	0.00 ± 0.29
C	Medium only	0.48 ± 0.36

JEH.3B. Although the yield of IL-2 and its mRNA was lower with this cell line than with EL4.E1, the effect of CsA was the same. When CsA was present during induction of IL-2, no translatable IL-2 mRNA was detected (Table 2). The mRNA's were also translated in wheat germ extract, with the same pattern of results as for EL4.E1 mRNA.

The translatability of various mRNA's used in these experiments, as assayed by the incorporation of [^{35}S]methionine into protein in wheat germ or rabbit reticulocyte lysates, was nearly the same. The general pattern of protein products synthesized, as analyzed by gel electrophoresis, was also similar. Other experiments indicate that CsA is highly selective in inhibiting IL-2 expression. Only a few (six to eight) proteins secreted by PMA-induced EL4 cells are sensitive to CsA, and analysis of cloned complementary DNA's indicates that less than 1 percent of mRNA's expressed by EL4 are sensitive to CsA. Thus CsA does not affect the yield or general character of mRNA from these cell lines, and the reduction of IL-2 mRNA levels is a specific effect.

Sequences coding for human IL-2 mRNA have been cloned (*12*). With cloned complementary DNA it is possible to measure IL-2 mRNA by hybridization, independent of its translatability. Messenger RNA isolated from JEH.3B cells stimulated with PMA and Con A was separated by gel electrophoresis under denaturing conditions, the gel was blotted onto nitrocellulose, and the blot was probed with cloned human IL-2 complementary DNA. A single band of the expected size (marker position, 12.5*S*) was detected in samples from



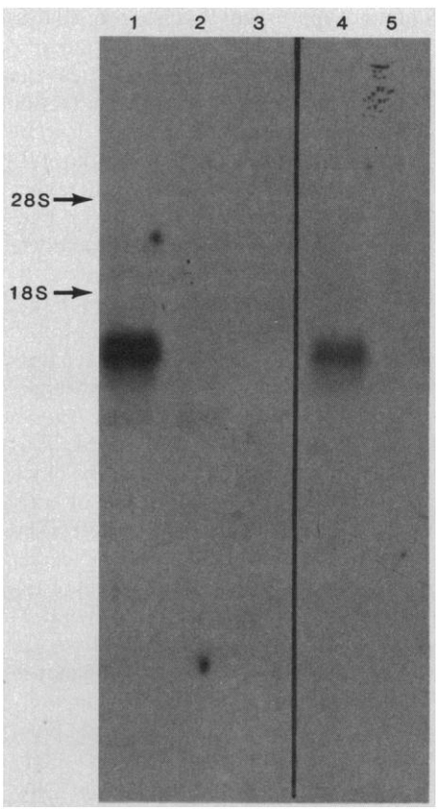


Fig. 1 (left). Effect of CsA on the production of IL-2 by murine EL4.E1 cells. (A) EL4.E1 cells were grown in RHFM (*7*) supplemented with 10 percent FCS, collected, and suspended at a density of 2×10^6 per milliliter in RHFM with 2 percent FCS and PMA (40 ng/ml). Portions (0.5 ml) of this suspension were added to an equal volume of medium containing 0 to 2000 ng of CsA per milliliter. Cells were cultured for 24 hours and the supernatants were collected by centrifugation and assayed for IL-2. A stock solution of CsA (1 mg/ml) (Sandoz lot OL 27-400N) was made up in dimethyl sulfoxide. Dimethyl sulfoxide at the concentrations added with CsA was found to have no effect on IL-2 production by EL4.E1 cells or its assay. Culture supernatants were assayed at several dilutions on the murine CTL line MTL2.8.2 by monitoring uptake of [^{125}I]iododeoxyuridine into cellular DNA (*8*). Activity was obtained from the titration curve and expressed as ED_{30} units (see legend to Table 1). The experiment was carried out in duplicate, with data from the two experiments indicated by (●) and (■). (B) Jurkat JEH-3B cells were grown in the same manner as the EL4.E1 cells and resuspended (2×10^6 cells per milliliter) in RHFM with 2 percent FCS, PMA (40 ng/ml), and Con A (66 μg/ml). Portions were mixed with equal volumes of CsA-containing medium and supernatants were harvested after 24 hours of incubation and assayed for IL-2 on the MTL2.8.2 line [human IL-2 is active on murine cells (*10*)]. Con A and PMA at equivalent concentrations had no effect on the bioassay. The experiment was carried out in duplicate. Fig. 2 (right). Absence of IL-2 mRNA in CsA-treated cells. Polyadenylated RNA was isolated from JEH.3B cells as described in the legend to Table 1, loaded onto a 1 percent agarose-formaldehyde gel, separated electrophoretically, and transferred to nitrocellulose (*13*). Hybridization to a cloned human IL-2 complementary DNA probe was carried out at 42°C in 50 percent formamide, 4× SSC (standard saline citrate), and 0.1 percent sodium dodecyl sulfate (SDS). Washing was done at room temperature in 2× SSC and 0.1 percent SDS and subsequently in 0.1× SSC and 0.1 percent SDS at the same temperature. Tracks 1 to 3 represent one experiment; tracks 4 and 5 another. Six micrograms of RNA was loaded in each lane. Jurkat JEH.3B cells were treated as follows: tracks 1 and 4, PMA and Con A; tracks 2 and 5, PMA and Con A in the presence of CsA (50 ng/ml); track 3, no stimulation.

stimulated cells. However, cells stimulated in the presence of CsA were negative for this band and did not show any hybridization (Fig. 2).

The combination of biological and chemical measurements for IL-2 mRNA makes it extremely unlikely that IL-2 mRNA was present in the CsA-inhibited cells in any form. We conclude that the accumulation of IL-2 mRNA seen after stimulation of Jurkat or EL4 cells is blocked by CsA. The applicability of these studies to normal human or murine lymphocytes must be confirmed by further experiments. It is important to note, however, that the effect of CsA on the production of IL-2 noted here with these tumor cell lines occurred at the same concentrations as with normal murine splenic lymphocytes stimulated with Con A (*2*). The CsA effect could be mediated by inhibition of IL-2 mRNA transcription or by its destabilization. It was recently shown that insulin selectively inhibits synthesis of mRNA for the enzyme phosphoenolpyruvate carboxykinase in a cell line (*14*); the present results would be explained if a similar inhibition of IL-2 mRNA synthesis were caused by CsA. In summary, it appears that at least part of the immunosuppressive activity of CsA is due to its ability to selectively inhibit accumulation of mRNA coding for IL-2 in human and murine T lymphocytes.

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Smell Identification Ability: Changes with Age

Abstract. *Smell identification ability was measured in 1955 persons ranging in age from 5 to 99 years. On the average, women outperformed men at all ages, and nonsmokers outperformed smokers. Peak performance occurred in the third through fifth decades and declined markedly after the seventh. More than half of those 65 to 80 years old evidenced major olfactory impairment. After 80 years, more than three-quarters evidenced major impairment. Given these findings, it is not surprising that many elderly persons complain that food lacks flavor and that the elderly account for a disproportionate number of accidental gas poisoning cases each year.*

The human sense of smell serves as an early warning system for the detection of fire, dangerous fumes, and polluted environments, and it largely determines the flavor and palatability of foods and beverages (1). Despite these important functions, the nature and degree of age-related changes in this sense are unknown. Practical considerations of stimulus presentation and control have limited human olfactory studies to only a few odorants and relatively small numbers of "young" and "old" subjects (unlike studies of vision and audition, where standardized tests have been administered to thousands of subjects of all ages). Although most olfactory threshold studies report higher thresholds in older than in younger subjects (*2*), some have not found age-related changes (*3*), and suprathreshold data on this topic are contradictory (*4*). As indicated in one recent review, "Most assume that aging is correlated with decreased ability in all sense modalities. Actually, the effect of aging on odor perception is not clear and has been debated since the beginning of this century" (*5*).

To date, no systematic evaluation of smell function has been performed across the entire age span. However, the recent development of a rapidly administered microencapsulated test of olfactory function has now made it possible to accurately assess the odor identification ability of large numbers of subjects in a standardized manner (*6*). Unlike detection threshold measures, this 40-odorant forced-choice test is sensitive to a broad range of olfactory deficits, including odor recognition problems originating in the central nervous system (*7*).

We now report, in a study of 1955 persons ranging in age from 5 to 99 years, that (i) average ability to identify odors reaches a peak in the third and fourth decades of life (between 20 and 40 years) and begins to decline monotonically after this time, (ii) a large proportion of elderly persons are anosmic, (iii) smoking has an adverse effect on odor identification ability, and (iv) women of all ages are generally more accurate than their male counterparts in identifying odors.

The subjects consisted of (i) employees of the University of Pennsylvania, (ii) residents of homes for the elderly, (iii) persons attending regional health fairs and other public events, (iv) university students, (v) primary and secondary grade school students, and (vi) youngsters enrolled at summer camps (*8*). Although the majority self-administered the test under our supervision, we administered it individually to many of the children and most of the elderly. In these instances, the response alternatives were read to the subjects before and during the sampling of each stimulus.

The sample quartiles for each decade are shown in Fig. 1. For the second through fifth decades, the data were modified according to a Box-Cox transformation, and a normal distribution was fit to the transformed values (*9*). For these decades the quartiles were calculated from the fitted normal distributions and then converted to the original scale of measurement.

A weighted least-squares multiple regression analysis revealed that age, gender, current smoking habits, and being a resident in a home for the elderly were significantly related to the test scores (*10*). On the average, women outperformed men at all ages (Fig. 1). Peak performance was achieved by both sexes during the third through fifth decades, followed by a slight decrease in average performance across the sixth and seventh decades and a marked decrease