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# TRANSLATION OF INTERLEUKIN 2 mRNA FROM HUMAN PERIPHERAL BLOOD LEUKOCYTES IN XENOPUS OOCYTES

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SUMMARY: Interleukin 2 was induced in cultures of human peripheral blood leukocytes by combined treatment with 12-O-tetradecanoylphorbol-13-acetate and a T-cell mitogen, Concanavalin A. Poly(A)-containing mRNA was isolated from these cultures and fractionated by sucrose density gradient centrifugation. When injected into Xenopus laevis oocytes, the mRNA preparation gave rise to interleukin 2 activity in the culture supernatant of the oocytes. The sucrose density gradient centrifugation analysis showed that the interleukin 2 mRNA sedimented at 10-12S, which suggests that it contains about 900-1,100 nucleotides.

Recently the regulation of immune reactions by soluble factors has been the subject of increasing interest. Morgan et al. (1) discovered a factor termed T-cell growth factor (TCGF) in mitogen-stimulated human lymphocyte conditioned media that allowed the long-term proliferation of human T-cells. It has been revealed that a number of lymphokines grouped separately in the past, such as costimulator, killer cell helper factor and T-cell replacing factor shared the common characteristics of molecules and biologic activities to TCGF (2). These lymphokines are now categorized under the single heading of interleukin 2 (IL-2) (2), and extensive studies have been reported on this group of lymphokines.

IL-2 permits the long-term culture and clonal derivation of normal T-cells without the loss of their functions. Various T-cell subsets and natural killer (NK) cells have been cloned by using IL-2 (3,4). IL-2 is not only essential for the proliferation of immunocompetent T-cells and NK cells

Abbreviations: IL-2, interleukin 2; PBL, peripheral blood leukocytes; TPA, 12-O-tetradecanoylphorbol-13-acetate; Con A, Concanavalin A; TCGF, T-cell growth factor; NK, natural killer; HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; FCS, fetal calf serum; SDS, sodium dodecyl sulfate.

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in vitro, but also effective on the proliferation of immunocompetent T-cells in vivo (5,6). IL-2 is produced in cultures of normal lymphocytes and T-cell lines after stimulation with T-cell mitogens (7,8). The addition of 12-O-tetradecanoylphorbol-13-acetate (TPA) to the mitogen-induced lymphocyte culture resulted in an apparent increase of IL-2 production (9). A number of investigations concerning IL-2 have been made so far, but much less information is available about IL-2 mRNA. Although several investigators have recently reported some characteristics of IL-2 mRNA isolated from mouse and primate T-cell lines (10,11) and that from human tonsilar lymphocytes (12), there have been no reports on IL-2 mRNA from human peripheral blood leukocytes (PBL).

In this paper we report the preparation for the first time of biologically active IL-2 mRNA from human PBL stimulated with TPA and Con A. We also show some characteristics of the isolated IL-2 mRNA.

## MATERIALS AND METHODS

Preparation of human PBL: Buffy coats from healthy donors (10-20) were pooled, and an equal volume of RPMI 1640 medium (M. A. Bioproducts, Maryland, U.S.A.) containing 100 μg/ml kanamycin (Takeda Chemical Industries, Ltd., Osaka, Japan), 2 mg/ml $NaHCO_3$, 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) was added to the buffy coat. After incubating the buffy coat overnight at 37°C, PBL were separated from the buffy coat by using dextran (13). The PBL thus obtained were washed with and resuspended in RPMI 1640 medium containing 10% fetal calf serum (M. A. Bioproducts, Maryland, U.S.A.) (FCS) at 5 x $10^6$ cells/ml.

Induction of IL-2: IL-2 was induced in the culture of PBL by combined treatment with TPA and Con A (P-L Biochemicals, Inc., Milwaukee, U.S.A.). In some experiments PBL were cultured with various concentrations of TPA and Con A in Linbro 24-well microplates (Flow Laboratories, Inc., Virginia, U.S.A.) in a volume of 1 ml under a humidified atmosphere of 5% $CO_2$ in air at 37°C. For the preparation of IL-2 mRNA, the PBL were treated with 15 ng/ml TPA and 40 μg/ml Con A, and the culture was performed in a volume of 3 liter using a spinner flask at 37°C for 21-24 hr. After incubation, the PBL were collected by centrifugation and immediately used for the extraction of total RNA.

Extraction and purification of IL-2 mRNA: The extraction of total RNA was performed according to the method of Kaplan et al. (14). Poly(A)-containing RNA was isolated from the total RNA by oligo(dT)-cellulose column chromatography following the method of Aviv and Leader (15). Subsequently, poly(A)-containing RNA was fractionated by the sucrose gradient (15-30%, w/v) centrifugation (16). As size markers, 23, 16 and 4S RNA (E. coli, Miles Laboratories, Inc., Indiana, U.S.A.) were sedimented in a parallel tube. Fractions were collected and the RNA in each fraction was precipitated by ethanol.

Translation of IL-2 mRNA: Translation of IL-2 mRNA was performed in Xenopus laevis oocytes (17). Twenty oocytes, microinjected with each RNA fraction (100 ng/oocyte), were cultured in 200 μl of Barth's medium at 24°C for 24

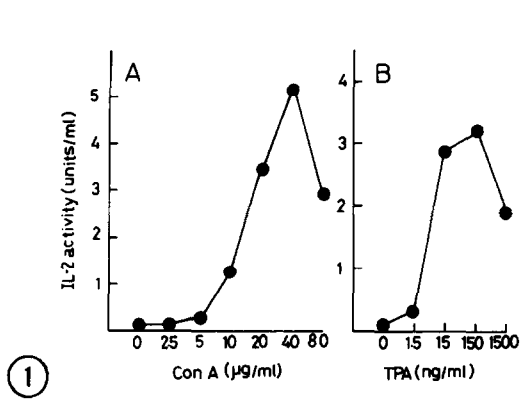
hr. After incubation, the supernatants were prepared by centrifugation at 15,000 x g and 4°C for 30 min.

Assay of IL-2: IL-2 dependent mouse NK cell line (NKC3) (18) was kindly provided by Dr. K. Kumagai of Tohoku University. IL-2 dependent human cell line was established from Con A-stimulated PBL (7). These cells were maintained in the presence of human IL-2 conditioned medium. The cells used for the assay were washed with and resuspended in RPMI 1640 medium containing 20% FCS at 6 x $10^5$ cells/ml. Test samples (50 µl) were serially diluted two fold on 96 well flat-bottomed microplates (A/S Nunc, Roskilde, Denmark), and the cell suspension (50 µl) was added to each well. After incubating the plates at 37°C for 20 hr, 1 µCi of [$^3$H]-thymidine (25 Ci/mmol, Radiochemical Center, Amsterdam, Denmark) was added to each well, and culture was performed for a further 4 hr according to the method of Gillis et al. (19). The amounts of [$^3$H]-thymidine incorporated were expressed as the mean c.p.m. of duplicate cultures. Units of IL-2 activity were determined by the probit analysis (19). One unit of IL-2 was arbitrarily defined as the amount of the activity present in a 48 hr cultured conditioned medium of human PBL (5 x $10^6$ cells/ml) in the presence of TPA (15 ng/ml) and Con A (20 µg/ml).

## RESULTS

Induction of IL-2 on PBL by stimulation with TPA and Con A: In order to determine the optimal doses of TPA and Con A for IL-2 induction, PBL were cultured with various concentrations of TPA and Con A, and the amounts of IL-2 in the supernatants were determined by using NKC3 cells. When PBL were precultured in the presence of TPA (15 ng/ml) for 3 hr, and subsequently various concentrations of Con A were added to the culture, the maximal production of IL-2 was observed at 40 µg/ml of Con A (Fig. 1A). On the other hand, in an experiment in which PBL were precultured with various concentrations of TPA for 3 hr and then Con A was added at 40 µg/ml, the maximal IL-2 production was obtained at 15-150 ng/ml of TPA (Fig. 1B). These results indicated that both TPA and Con A were essential for the production of IL-2 by PBL.

In the presence of 15 ng/ml of TPA and 40 µg/ml of Con A, the amount of IL-2 increased linearly from the beginning of culture to 72 hr and reached a plateau at this point. The concentration of IL-2 after 72 hr of culture was 2.5-3.0 units/ml (data not shown ). The kinetics of IL-2 production in a large scale culture (3 liter spinner flask) was substantially similar to that in a small scale culture (1 ml Linbro microplate). Total RNA was extracted from PBL at 21-24 hr after the addition of TPA and Con A.



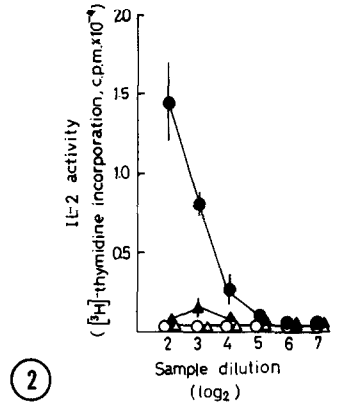




Figure 1. Effect of Con A and TPA concentrations on IL-2 production by PBL. (A), PBL (5 x $10^6$ cells/ml) were precultured with TPA (15 ng/ml) for 3 hr in a Linbro 24-well microplate. Various concentrations of Con A were then added to the culture and incubated for 48 hr. (B), PBL (5 x $10^6$ cells/ml) were precultured with various concentrations of TPA for 3 hr in a Linbro 24-well microplate. Subsequently Con A (40 μg/ml) was added to the culture and incubated for 72 hr. After incubation, these culture supernatants were collected and assayed for IL-2 activity by using NKC3 cells.

Figure 2. IL-2 producing activity of poly(A)-containing RNA sedimented on sucrose density gradient centrifugation in Xenopus laevis oocytes. PBL (1.5 x $10^{10}$ cells) were cultured in the presence of TPA (15 ng/ml) and Con A (40 μg/ml) at 37°C for 21 hr in a volume of 3 liter using a spinner flask. Then PBL were collected and total RNA was extracted. Poly(A)-containing RNA was isolated from the total RNA by oligo(dT)-cellulose column chromatography. The poly(A)-containing RNA was subsequently fractionated by sucrose density gradient centrifugation. RNA sedimented below 18S was collected and microinjected into Xenopus laevis oocytes. After incubation of oocytes in the Barth's medium, the culture supernatant or supernatant of homogenate of oocytes were prepared and tested for the IL-2 activity on NKC3 cells. (●): Culture supernatant of oocytes injected with RNA. (○): Culture supernatant of oocytes injected with distilled water. (▲): Supernatant of homogenate of oocytes injected with RNA (△): Supernatant of homogenate of oocytes injected with distilled water.

Isolation and analysis of IL-2 mRNA by sucrose density gradient centrifugation: The mRNA isolated from TPA and Con A-stimulated PBL and sedimented on sucrose density gradient centrifugation was divided into two fractions, RNA fractions at the region of 18-23S and those below 18S. The culture supernatant of oocytes injected with RNA that sedimented below 18S contained an apparent IL-2 activity on NKC3 cells (Fig.2). However, RNA sedimented at 18-23S were unable to induce IL-2 activity (data not shown). As shown in Fig. 2, the supernatant fraction from homogenates of oocytes injected with the RNA sedimented below 18S also exhibited little IL-2 activity. Our preliminary experiments revealed that the homogenate of oocytes contained some substance(s) toxic for NKC3 cells.

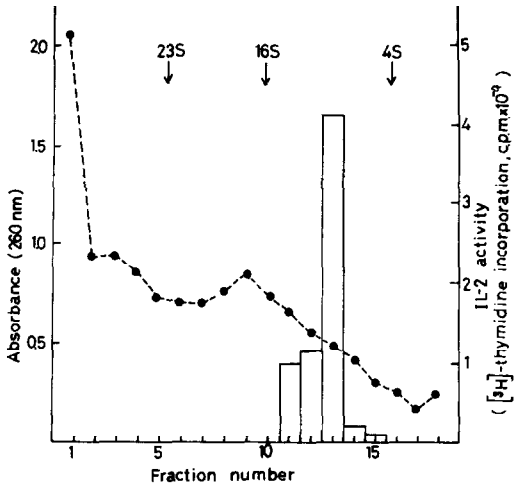



Figure 3. Sedimentation of IL-2 mRNA on sucrose density gradient centrifugation. Poly(A)-containing RNA was isolated from total RNA extracted from PBL which were cultured in the presence of TPA (15 ng/ml) and Con A (40 μg/ml) for 24 hr. Subsequently poly(A)-containing RNA was centrifuged on a sucrose gradient (15-30%, w/v) at 25,000 r.p.m., at 20°C for 21 hr and the RNA sedimented was divided into 18 fractions. Each RNA fraction sedimented below 18S was used to measure the IL-2 mRNA activity by injecting it into Xenopus laevis oocytes. The culture supernatants of oocytes were diluted four fold and assayed for IL-2 activity by using NKC3 cells. The column in the figure indicates the IL-2 activity on NKC3 cells. (●): Absorbance at 260 nm of each RNA fraction. The arrows indicate the positions of standard RNAs sedimented in a parallel tube.

In order to determine the precise sedimentation coefficient of the IL-2 mRNA, poly(A)-containing RNA was sedimented on sucrose density gradient centrifugation and 18 fractions were collected. Each RNA fraction sedimented below 18S was injected into oocytes and assayed for IL-2 producing activity. As shown in Fig. 3, RNA fractions sedimented at 9-15S induced IL-2 activity in the culture supernatant of oocytes and the peak activity was observed at 10-12S. The same active fractions showed the activity on IL-2 dependent human cells as well as NKC3 cells (data not shown).

## DISCUSSION

In this paper we have reported the isolation and partial characterization of the IL-2 mRNA prepared from human PBL. This is believed to be the first example of the isolation of IL-2 mRNA from human PBL. The sedimentation coefficient of the IL-2 mRNA suggests that it contains 900-1,100 nucleotides capable of coding for a protein with a molecular weight of as high as 30,000 Recently, it was reported that the molecular weights of native

IL-2 purified from human normal lymphocytes and a T-cell line were 14,000-16,200 and 13,500 daltons, respectively, by SDS-polyacrylamide gel electrophoresis (20,21). The IL-2 mRNA seems to be much larger than expected from these molecular weights.

The sedimentation coefficient of IL-2 mRNA derived from EL-4, a mouse T-cell line, was 11-12S, and that from MLA 144, a marmoset T-cell line, 14-16S (10,11). Furthermore, IL-2 mRNA from human tonsilar lymphocytes was reported to be about 10-13S (12). These values agree well with our results. The IL-2 mRNA from human tonsilar lymphocytes stimulated with phytohemagglutinin sedimented to give two peaks of IL-2 mRNA activity, a major peak with about 10S and a minor peak with about 13S (12). In this report, however, the IL-2 mRNA isolated from human PBL stimulated with TPA and Con A sedimented to give a single peak of 10-12S. Several investigators have described the heterogeneity of IL-2 molecules (21,22). Robb and Smith (22) described that IL-2 molecule produced by human tonsilar lymphocytes might be a single, variably glycosylated polypeptide and not a class of unrelated molecules. Our result seems to be consistent with their assumption. However, the possibility remains that heterogeneous IL-2 molecules may be produced by the lymphocytes from different sources; or heterogeneity may be attributable to differences of the methods for IL-2 induction.

The species specificity of IL-2 obtained from different animals is rather limited; IL-2 from mice is active on mouse cells but inactive on rat and human cells, IL-2 from rat is active on both rat and mouse cells but inactive on human cells, however, human IL-2 is active on human, rat and mouse cells (23). Robb *et al.* (24) demonstrated that the homogeneous human IL-2 molecule was able to bind to IL-2 dependent mouse cells, and they suggested that human IL-2 was active on both human and mouse cells through the common mode of action. Consistent with their results, the IL-2 translated from our mRNA was active on both human and mouse IL-2 dependent cell lines.

The preparation of IL-2 mRNA will allow the synthesis and cloning of IL-2 cDNA. The sequence analysis of the cDNA encoding IL-2 must reveal the details of the structure of IL-2 molecule(s).

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