Journal of Cellular Biochemistry 60:495–507 (1996)

Expression of Human Mitochondrial NADP-Dependent Isocitrate Dehydrogenase During Lymphocyte Activation

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Abstract In the process of identifying genes involved in optimization of lymphocyte activation, we have cloned the human mitochondrial NADP-dependent isocitrate dehydrogenase (mNADP-IDH) cDNA. The cDNA and its deduced amino acid (AA) sequence had a high degree of homology with those of the porcine and bovine. The heart and muscle had the highest constitutive expression of the gene. The expression of steady-state mRNA in the resting T and B lymphocytes was low but was induced after mitogen stimulation. The mRNA levels peaked around 48 h and remained elevated at 72 h. At the protein level, the micothondrial but not cytosolic NADP-IDH activity was augmented after the mitogen stimulation. There was no cell cycle–dependent fluctuation of mNADP-IDH expression in synchronized Jurkat cells. In T and B cells, rapamycin (RAPA) could repress the mitogen-stimulated mNADP-IDH expression, although most of the early or late phase activation-related genes including a G-protein β subunit–related gene H12.3 were not affected by the drug. The restricted expression of the gene in certain tissues and the activation-related expression in lymphocytes suggest that this gene might be necessary for optimal functions in heart, muscle, and the activated lymphocytes.

Key words: E.C.1.1.1.42, molecular cloning, NADP-IDH cDNA rapamycin, cyclin E

Cell cycle entry is a tightly controlled event with a major restriction point in the late G_1 phase, and this point is termed START in budding yeast [Hartwell et al., 1974]. In yeast, START is probably controlled by a fairly small and specific family of regulatory proteins, mainly kinases and phosphatases [Hunt et al., 1993]. The passage of the G_1 control point is necessary but not sufficient for the initiation of chromosome replication, as demonstrated in the case of yeast *Saccharomyces cerevisiae* [Hunt et al., 1993]. Additional gene products must be present to sustain and optimize the cascade of molecular events leading to cell proliferation. The gene products in this category include, among other things, enzymes for energy supply and for synthesis of metabolites in order to meet the increasing requirement during cell replication [Johnson, 1992].

After appropriate activation, resting lymphocytes in the G_0 phase will traverse through the G_1 phase and enter the S phase. In lymphocytes as well as in other mammalian cells, the entry control and optimization of cell cycling involve more complicated regulation compared to yeast, and de novo mRNA transcription and protein synthesis are essential [Adolph et al., 1993]. At the present time, our knowledge about the molecular events of the cascade from lymphocyte activation to proliferation is limited. We elect to identify mitogen-inducible genes in the lymphocytes with a view to dissecting the cascade. In so doing, we have cloned the human cDNA of mitochondrial NADP-dependent isocitrate dehydrogenase (mNADP-IDH), which has not been previously sequenced. The mNADP-IDH cDNA and its deduced amino acid (AA) sequence had a high degree of homology with those of the porcine and bovine. The expression of human mNADP-IDH was augmented in the late G_1 phase after lymphocyte activation, but it was not cell cycle–

Abbreviations used: cNADP-IDH, cytosolic NADP-dependent IDH; IDH, isocitrate dehydrogenase; mNADP-IDH, mitochondrial NADP-dependent IDH; NAD-IDH, NAD-dependent IDH.

Received July 25, 1995; accepted August 24, 1995.

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dependent. Several lymphokines such as IL-2, IL-4, IFN-γ, and IL-6, while known to increase the mitogen-induced T and B cell proliferation, did not further enhance the mNADP-IDH expression. The human mNADP-IDH was sensitive to rapamaycin. The role of mNADP-IDH in lymphocyte activation was discussed.

MATERIALS AND METHODS

Reagents

Staphylococcus aureus Cowan I (SAC) was purchased from Calbiochem (La Jolla, CA) and Lymphoprep from NYCOMED (Oslo, Norway). RPMI 1640, fetal calf serum (FCS), penicillin-streptomycin, and L-glutamine were ordered from GIBCO/BRL (Gaithersburg, MD). The random primer labeling kit was from Pharmacia-LKB (Baie d'Urfe, Quebec, Canada), [α-^{32}p]-dCTP was from ICN (Mississauga, Ontario, Canada), and [α-^{35}S]-dATP was from Amersham (Arlington Heights, IL). The Librarian I cDNA library construction system and the Subtractor I kit were purchased from Invitrogen (San Diego, CA). The Sequenase 2.0 kit was purchased from U.S. Biochemical Corp. (Cleveland, OH).

Cell Culture

Human tonsillar T and B cells were prepared as described before [Luo et al., 1992]. The T cells were obtained by one-cycle sheep red blood cell (SRBC) rosetting, and such preparations contained about 90–93% CD3$^+$ cells. The T cell depleted fraction was referred to as B cells, which contained more than 90% CD20$^+$ cells. In some experiments, these B cells were further fractionated by percoll density gradients, and the high density B cells were isolated from the interface of 65% and 55% Percoll. All the cells were cultured in RPMI 1640 supplemented with 10% FCS, L-glutamine, and antibiotics.

Construction of cDNA Library of Activated B Cells

The high density tonsillar B cells were activated by SAC (1:10,000 dilution) for 16 h, and the total RNA was prepared by the guanidine/CsCl method [Ausubel et al., 1991]. Poly-A$^+$ RNA (twice selected on oligo-dT columns) mRNA was reverse-transcribed for the first strand cDNA using oligo-dT as primers. After second strand synthesis, the cDNA was blunt-ended, ligated with nonpalindromic BstXI linkers, and cloned into the BstXI site of the eukaryotic expression vector pcDNA I. The detailed procedure for the library construction was described in the manufacturer's instructions (Invitrogen). Approximately 0.3 × 10^6 independent clones were obtained.

Preparation of a Subtracted cDNA Probe

Messenger RNA was prepared from the SAC-activated (16 h) high density B cells and reverse-transcribed using random primers and [α-^{32}P]dCTP. The radiolabeled cDNA probe was subtracted with a tenfold excess of mRNA from high density B cells using a Subtractor I kit from Invitrogen according to the manufacturer's instructions.

Screening of cDNA Libraries

The cDNA library of human activated B cells was screened with subtracted cDNA probes prepared as described above. To obtain full-length mNADP-IDH cDNA, λgt10 human heart cDNA library (Clontech, Palo Alto, CA) was screened with the 0.8 Kb mNADP-IDH cDNA probe, which was ^{32}P-labeled with random primers. The detailed screening protocols were described by Ausubel et al. [1991].

DNA Sequencing

cDNA was sequenced with the chain termination method on double-stranded plasmid DNA using Sequenase 2.0 and [α-^{35}S]dATP according to the manufacturer's instructions.

Northern Blot Analysis

Total lymphocyte RNA and tissue RNA were electrophoresed on 0.85% agarose/formaldehyde gels, and RNA loading (5–15 μg/lane, consistent within a given experiment) was monitored by the intensity of 18S and 28S bands of ribosomal RNA after ethidium bromide staining. The RNA was transfered onto Biotrans nylon membranes (ICN) and crosslinked with UV irradiation. The membranes were hybridized at 42°C overnight with the mNADP-IDH cDNA probe. The final wash of the membranes was at 56°C for 30 min with 0.1 × SSC buffer (15 mM NaCl, 1.5 mM Na_3 citrate) containing 0.1% SDS.

Assays for IDH Activity

The NADP-IDH activity was assayed as described by Bergmeyer et al. [1983]. The organ mitochondrial fraction was prepared according

to Guerra [1974]. Briefly, the tissues were first homogenized with Dounce homogenizer and were centrifuged at 800*g* to remove cell debris. The homogenate was then centrifuged at 13,000*g* for 30 min to pellet the mitochondrial fraction, while the supernatant represented the cytosolic fraction. For lymphocyte mitochondrial preparation, the cell membrane was solubilized with 2 mM digitonin [Zuurendonk et al., 1979], and the fraction of mitochondria, the membrane of which is resistant to digitonin, was pelleted by centrifugation at 13,000*g* for 5 min. The supernatant represented the cytosolic fraction. The mNADP-IDH activity was corrected for the cross-contamination between the cytosolic and mitochondrial fractions, according to the activities of the exclusive cytosolic LDH (lactate dehydrogenase) and of the exclusive mitochondrial GLDH (glutamate dehydrogenase) [Guerra, 1974; Ragan, 1983]. The correction formula is as follows:

$$\text{mIDH} \times \text{mProtein} = (c \times a) + [M \times (\text{mProtein} - a)]$$

$$\text{cIDH} \times \text{cProtein} = (M \times b) + [C \times (\text{cProtein} - b)]$$

where

$$a = \frac{\text{mLDH}}{\text{cLDH}} \times \text{mProtein}$$

and

$$b = \frac{\text{cGLDH}}{\text{mGLDH}} \times \text{cProtein}.$$

mIDH, mLDH, and mGLDH stand for measured IDH, LDH, and GLDH activities (μ/mg/min) in the mitochondrial fraction, respectively, while cIDH, cLDH, and cGLDH stand for those in the cytosolic fraction. mProtein and cProtein represent protein concentrations in the mitochondrial and cytosolic preparations, respectively. C and M are the corrected mitochondrial and cytosolic IDH activity, respectively. The contamination of mitochondrial protein to the cytosolic protein was normally below 15%, while the cytosolic to the mitochondrial below 35%.

Synchronization of Jurkat Cells

Jurkat cells were first cultured for 24 h in isoleucine-deficient RPMI 1640 medium supplemented with 10% extensively dialyzed FCS. The cells were then cultured in complete RPMI 1640 medium in the presence of 1 nM hydroxyurea for 16 h. As a consequence, these cells were synchronized and blocked at the G_1/S boundary. The blockage was released by washing out hydroxyurea, and the cells were then cultured in complete medium. The cells were sampled at different time points after the release for flow cytometry and Northern blot analyses.

Cell Cycle Analysis

Flow cytometry was employed for cell cycle analysis. Jurkat cells were first fixed with cold 70% ethanol for 1 h at 4°C. Followed by two washes with PBS, the cells were stained with propidium iodide at room temperature for 30 min in a solution containing propidium iodide (20 μg/ml), RNase A (20 μg/ml), sodium citrate (0.1%), and NP-40 (0.37%). Five thousand viable lymphocytes (gated by forward and side scattering) were then analyzed in a Coulter Profile I flow cytometer (Coulter, Hialeah, FL). The computer program Elite was employed for data analysis.

RESULTS

cDNA Library Screening

In order to identify genes which were induced in late G_1 phase of lymphocyte activation, a plasmid cDNA library of activated B cells was screened with a probe made from cDNA of activated B cells subtracted with mRNA of resting B cells. About 10% of the clones screened were positive, and 150 of the positive clones were partially sequenced. After excluding those clones with inserts derived from ribosomal RNA, repetitive elements, poly-A tails, and other known genes, 15 clones were selected. The inserts of these clones were used as probes in Northern blot analysis of the total RNA of resting or mitogen-activated T cells in order to identify activation-related genes common to both T and B cells. Nine of the 15 genes had detectable expression. Among the nine, the expression of clone #256 along with other four was upregulated after the mitogen stimulation. We elected to further characterize clone #256.

The insert in clone #256 was about 0.8 Kb and had a high degree of homology with cDNA of porcine mNADP-IDH (89.6% indentity in 625 bp overlap [Haselbeck et al., 1992]). This insert was then used as a probe to screen human λgt10 heart cDNA library, since the heart had the highest expression of the mNADP-IDH mRNA

(refer to following sections). Positive clones with the longest inserts were cloned into the EcoRI site of pGEM4 and then sequenced.

DNA Sequence Analysis of the mNADP-IDH cDNA

The cDNA of the human mNADP-IDH was sequenced on both strands. The cDNA sequence (submitted to Genbank and EMBL) and deduced AA sequence were analyzed with programs of Genetics Computer Group. The sequence databanks from GenBank (version 85, October 94), EMBL (version 40, September 94), Swissprot (version 30, October 94) and PIR protein (version 42, September 94) were employed. The cDNA was 1,596 bp long and contained an open-reading frame (ORF) of 419 amino acids (Fig. 1). The coding sequence of the cDNA shared a high degree of homology with the porcine mNADP-IDH cDNA (91.9% in 1,140 bp overlap [Haselbeck et al., 1992]) and with the bovine cDNA (91.0% in 1,140 bp overlap [Hul et al., 1993]). The deduced AA sequences (Fig. 2) of the mNADP-IDH protein was also highly conserved when compared with the porcine (96.1% homology in 381 AA overlap) or with the bovine (95.8% homology in 381 AA overlap).

The human protein had seven out of seven conserved cysteine residues (Fig. 2, bold C). One of the cysteine-containing regions, DLAGCIHGLSNVK (AA residues 414–426) (Fig. 2), was 100% conserved among the human, bovine, and porcine, and this region has been implicated in the formation of NADP-binding pocket according to affinity labeling of the peptide with a reactive NADP analogue [Bailey et al., 1987]. In E. coli mNADP-IDH, seven AA residues (i.e., Arg 119, Arg 153, Tyr 160, Lys 230, Asp 307, Asp 311, and Glu 336) are implicated in binding of isocitrate plus Mg^{++} according to crystallographic analysis [Hurley et al., 1991]. In the human protein, all the seven corresponding residues (i.e., Arg 140, Arg 172, Tyr 179, Lys 251, Asp 314, Asp 318, and Glue 343 or Glu 345) were conserved. The human protein had two conserved N-linked glycosylation sites on Asn 136 and Asn 433 (Fig. 2).

Expression of mNADP-IDH in Different Tissues

The 0.8 Kb human cDNA fragment was used as a probe in Northern blot analyses for the human tissue samples (Fig. 3). The heart and muscle had a very high mRNA expression, and this enzyme probably plays an important role in these organs. In all the other tissues examined, the expression of the steady-state mRNA was low or undetectable. Most of the malignant tumors or tumor derived cell lines, such as colo-201 and colo-205 cells from colon tumors, MCF-7 from a breast adenocarcinoma, H596 from a lung adenosquamous carcinoma, Jurkat from a T cell leukemia, and U937 from a histiocytic lymphoma, expressed medium to high levels of the mRNA. However, HeLa cells derived from a cervix carcinoma, A-253 from a maxillary gland epidermal carcinoma, and RVH cells from a large cell lung carcinoma had no levels of the expression, although these cells underwent vigorous growth. This suggests that this gene expression is not inalienably coupled to cell proliferation. We have also assayed the mNADP-IDH activity in the mitochondrial preparations of different tissues, and the enzymatic activities were in agreement with their respective mRNA levels. (Fig. 3B).

Expression of mNADP-IDH in Lymphocytes

Next, the expression of the mNADP-IDH gene in resting and activated lymphocytes was investigated. In T cells stimulated with PHA, the steady-state mRNA was induced after 20 h and peaked around 40–48 h. (Fig. 4A,B). Similar upregulation was also found in SAC-activated tonsillar B cells (Fig. 4A,C).

The enzyme activities in these lymphocytes were consistent with their mNADP-IDH mRNA levels. In the mitochondrial preparation of human tonsillar cells, the enzymatic activity increased 2.5–3.5-fold in terms of either activity per milligram of protein (Fig. 5A) or activity per cell (Fig. 5B) 48 h after PHA (phytohemagglutinin) and PWM (pokeweed mitogen) stimulation. It was interesting to note that the cytosolic NADP-IDH activity remained below the detectable level before and after mitogen stimulation and was at least tenfold lower than that of the mNADP-IDH.

We have also examined the effect of several lymphokines (e.g., IL-2, IL-4, IFNγ, and IL-6, which are known to augment the mitogen-stimulated lymphocyte proliferation [Okada et al., 1979; Wagner et al., 1978; Paul 1991; Hirano et al., 1987]) on the gene expression of mNADP-IDH. IL-2 and IL-4 by themselves had

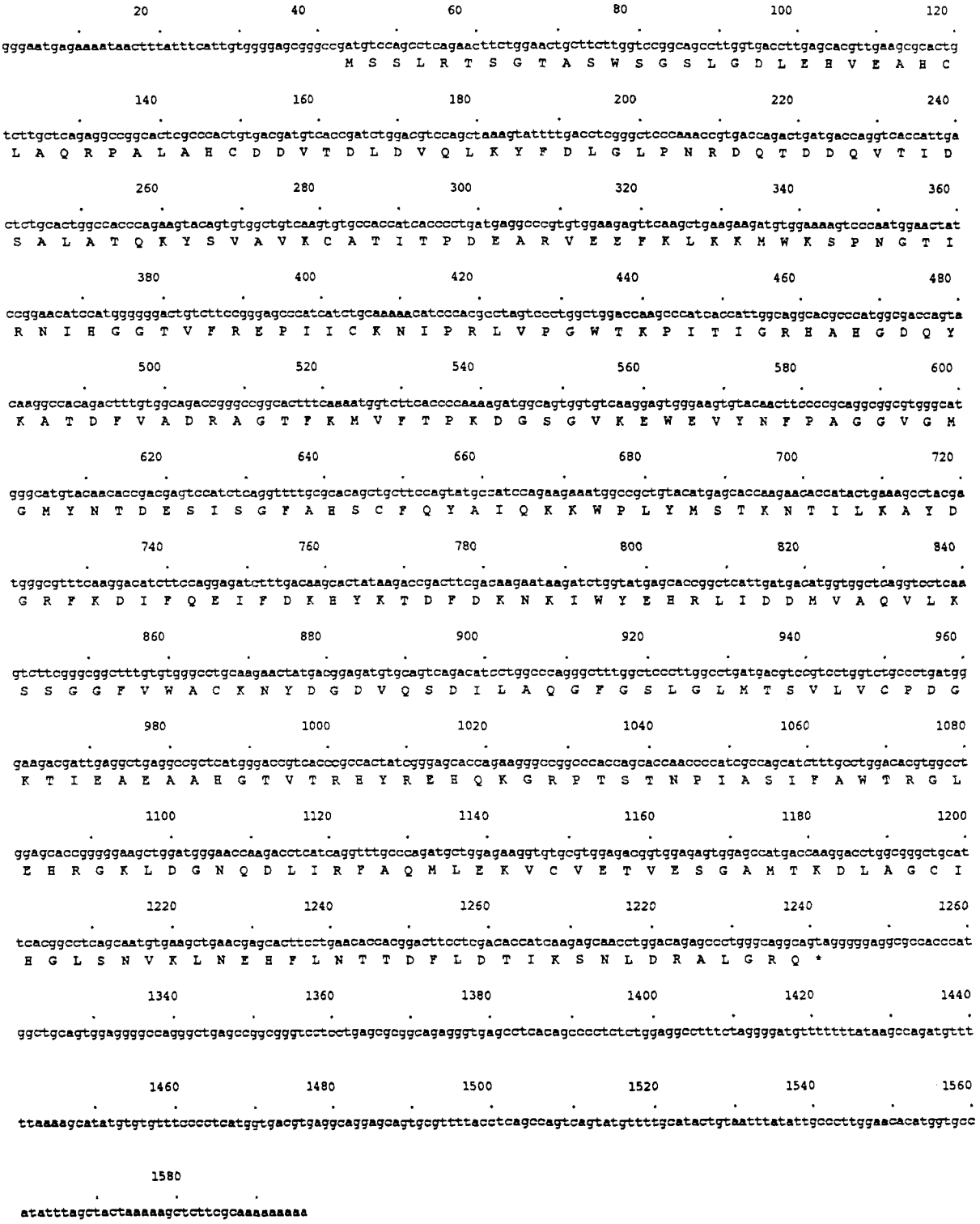

Fig. 1. The cDNA and AA sequences of the human mNADP-IDH.

```
          1                                                  50
Human     .......... ..MSSLRTSG TASWSGSLGD LEHVEA.HCL AQRPALA...
Bovine    MAGYLRVVRS LCRA-GSG-A W-PRALTAPN -QEQPRR-YA DK-IKV-KPV
Porcine   .......... .......... .......... .ARAA-R-YA DQ-IKV-KPV

          51                                                 100
Human     ...HCDDVT. .......... ...DLDVQLK YFDLGLPNRD QTDDQVTIDS
Bovine    VEMDGDEM-R IIWQFIKEKL ILPHV----- ---------- --N-------
Porcine   VEMDGDEM-R IIWQFIKEKL ILPHV----- ---------- --N-------

          101                                                150
Human     ALATQKYSVA VKCATITPDE ARVEEFKLKK MWKSPNGTIR NIHGGTVFRE
Bovine    ---------- --C------- ---------- -----N---- --L-------
Porcine   ---------- --C------- ---------- -----N---- --L-------

          151                                                200
Human     PIICKNIPRL VPGWTKPITI GRHAHGDQYK ATDFVADRAG TFKMVFTPKD
Bovine    ---C------ ---------- ---------- -----V---- ---V------
Porcine   ---C------ ---------- ---------- -----V---- ---I------

          201                                                250
Human     GSGVKEWEVY NFPAGGVGMG MYNTDESISG FAHSCFQYAI QKKWPLYMST
Bovine    ---P------ ---------- ---------- ----C----- ----------
Porcine   --SA-Q---- ---------- ---------- ----C----- ----------

          251                                                300
Human     KNTILKAYDG RFKDIFQEIF DKHYKTDFDK NKIWYEHRLI DDMVAQVLKS
Bovine    ---------- -------A-- E-----E--- H--------- ----------
Porcine   ---------- ---------- E--------- Y--------- ----------

          301                                                350
Human     SGGFVWACKN YDGDVQSDIL AQGFGSLGLM TSVLVCPDGK TIEAEAAHGT
Bovine    -------C-- ---------- ---------- -----C---- ----------
Porcine   -------C-- ---------- ---------- -----C---- ----------

          351                                                400
Human     VTRHYREHQK GRPTSTNPIA SIFAWTRGLE HRGKLDGNQD LIRFAQMLEK
Bovine    ---------- ---------- ---------- ---------- ------T---
Porcine   ---------- ---------- ---------- ---------- ------T---

          401                                                450
Human     VCVETVESGA MTKDLAGCIH GLSNVKLNEH FLNTTDFLDT IKSNLDRALG
Bovine    -C-------- -------C-- ---------- --N-S----- ----------
Porcine   -C-------- -------C-- ---------- --N-S----- ----------

          451
Human     RQ*
Bovine    Q-*
Porcine   --*
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Fig. 2. Peptide sequence homology of mNADP-IDH of different species. Boldface C and N represent cysteines and N-linked glycosylation sites, respectively.

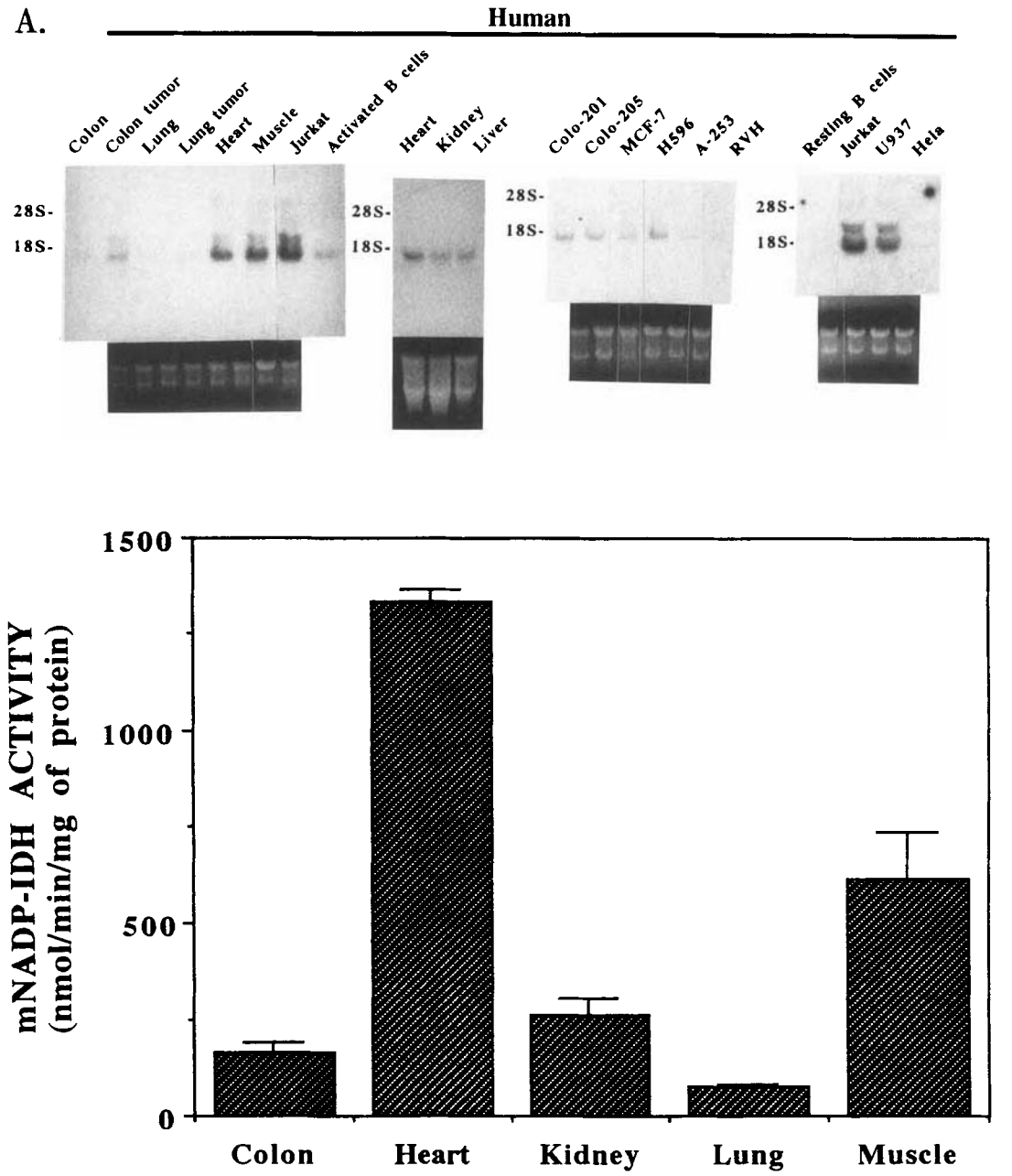

Fig. 3. Tissue-specific expression of mNADP-IDH. **A:** Northern blot analysis of mNADP-IDH expression in different human tissues. Loading of the total RNA is shown in the lower panels by ethidium bromide staining of the 18S and 28S ribosomal RNA. **B:** mNADP-IDH activity in the mitochondrial preparation of different human tissues. The plots represent results (mean ± 1 SD) of two experiments, and the data have been corrected for cross-contamination between cytosolic and mitochondrial proteins.

no effect on the gene expression and did not further enhance the PHA-stimulated expression in tonsillar T cells (Fig. 4B). IFNγ and IL-6 did not further enhance the mNADP-IDH gene expression in SAC-stimulated tonsillar B cells (Fig. 4C).

mNADP-IDH Expression Was Not Periodic During the Cell Cycle

We have noticed that the mNADP-IDH gene expression peaked around 40–48 h in mitogen-stimulated T and B cells (Fig. 4A,B). We won-

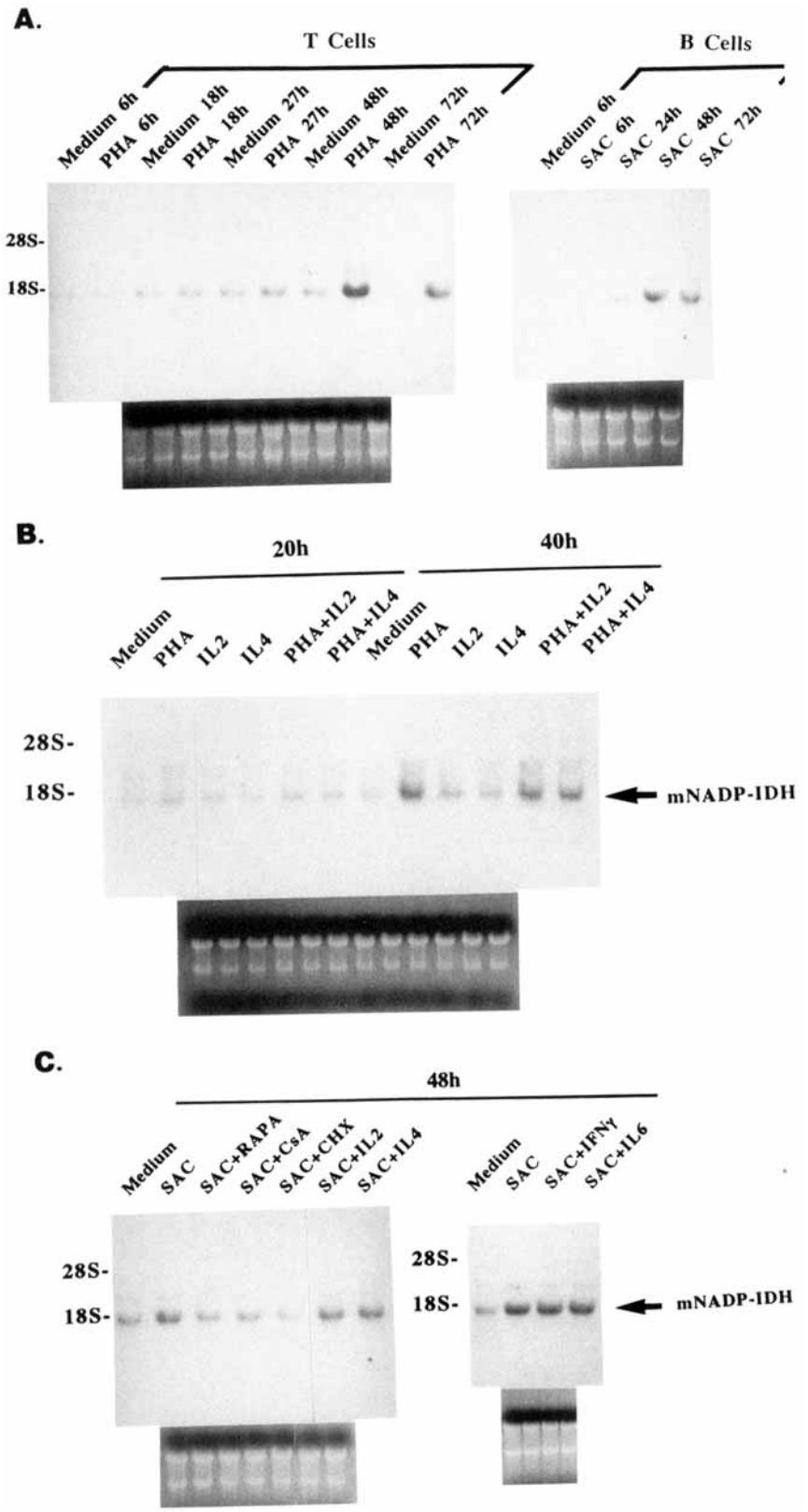

Fig. 4. Northern blot analysis of mNADP-IDH gene expression in lymphocytes. **A,B:** Total RNA from human tonsillar T or B cells cultured in the presence of various reagents (PHA, 2 μg/ml; SAC, 1:10,000 dilution; IL-2, 25 U/ml; IL-4, 15 ng/ml) as indicated. **C:** Total RNA from human tonsillar B cells cultured for 48 h in the various reagents as indicated (SAC, 1:10,000 dilution; IFNγ, 1,000 U/ml; IL-6, 100/ml; RAPA, 10 nM; CsA, 1 μM; cyclohexamide (CHX), 10 μg/ml).

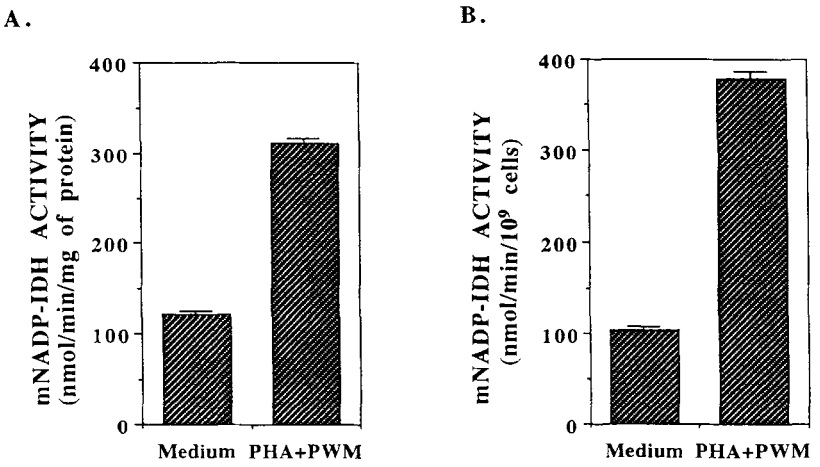

Fig. 5. mNADP-IDH activity in mitochondrial preparation of resting and activated lymphocytes. Human tonsillar total cells were stimulated with PHA (2 μg/ml) and pokeweed mitogen (PWM) (0.25%) for 48 h, and the mNADP-IDH activity in the mitochondrial preparation was assayed. The results are presented either at per milligram of protein (**A**) or per 10^9 cells (**B**). The cross-contamination between the cytosolic and mitochondrial proteins was corrected. The results of representative experiments are shown, and similar results were obtained in two other experiments.

dered whether this gene had a periodic expression and was required for a certain phase during the cell cycle, because most of the mitogen-stimulated cells were at late G_1 phase during this period. A model of synchronized Jurkat cells was established by isoleucine starvation plus hydroxyurea blockage. More than 80% of the cells were at the G_1/S boundary at the time of hydroxyurea release (0 h) (Fig. 6A). At 6 and 12 h after the release, the peak shifted to the S and G_2/M phases, respectively. At 15 h, most of the cells reentered the G_1 phase, and after 24 h the synchronization was no longer apparent. In this model, cyclin E had a typical G_1-dependent expression (Fig. 6B). The signals were high at 0 h and 18 h when most of the cells were at G_1, while almost undetectable at 9 h when most of the cells were at S and G_2/M phase. This supports the role of cyclin E in cell cycle control and is consistent with the previous report that cyclin E has cell cycle–dependent expression in other mammalian cells like HeLa [Lew et al., 1991]. However, mNADP-IDH showed no decrease in S and G_2/M phase.

Effect of Immunosuppressants on the mNADP-IDH Expression

Immunosuppressants rapamycin (RAPA) and cyclosporin A (CsA) can strongly inhibit mitogen-stimulated T cell proliferation [Morris, 1992]. We first examined the effect of these two drugs on the mNADP-IDH expression in human T cells. Both RAPA and CsA could inhibit the mitogen-induced mNADP-IDH expression in T cells, as shown in Figure 7A,B. The inhibition was obvious between 24 h and 72 h. As internal controls, the same membranes were hybridized with the H12.3 cDNA which is a mitogen-inducible G protein β subunit–related gene [Shan et al., 1994]. Although CsA could inhibit H12.3 at 40 h, RAPA had no apparent effect on it. It is worth mentioning that RAPA at 100 nM, which was strongly inhibitory to Jurkat proliferation (Kuo et al., 1991; our unpublished observation) had no obvious effect on the expression of either cyclin E or mNADP-IDH in synchronized Jurkat cells (Fig. 6B). Additional study in SAC-activated B cells showed that the mNADP-IDH expression in these cells was also sensitive to both CsA and RAPA (Fig. 4C).

DISCUSSION

There are three isoforms of IDH in mammalian cells. The NAD-dependent IDH (NAD-IDH) (EC.1.1.1.41) is present only intramitochondrially. The other two are NADP-dependent (EC.1.1.1.42), one being cytosolic (cNADP-IDH) and the other being in the mitochondrial matrix (mNADP-IDH). It is now known that the two mitochondrial IDH isoforms (i.e., NAD-IDH and mNADP-IDH) are coded by different genes [Cupp et al., 1991; Cupp et al., 1992], and the cytosolic and mitochondrial NADP-dependent IDH (i.e., cNADP-IDH and mNADP-IDH) are immunogically distinct [Jennings et al., 1991]. While the NAD-dependent IDH plays a pivotal role in

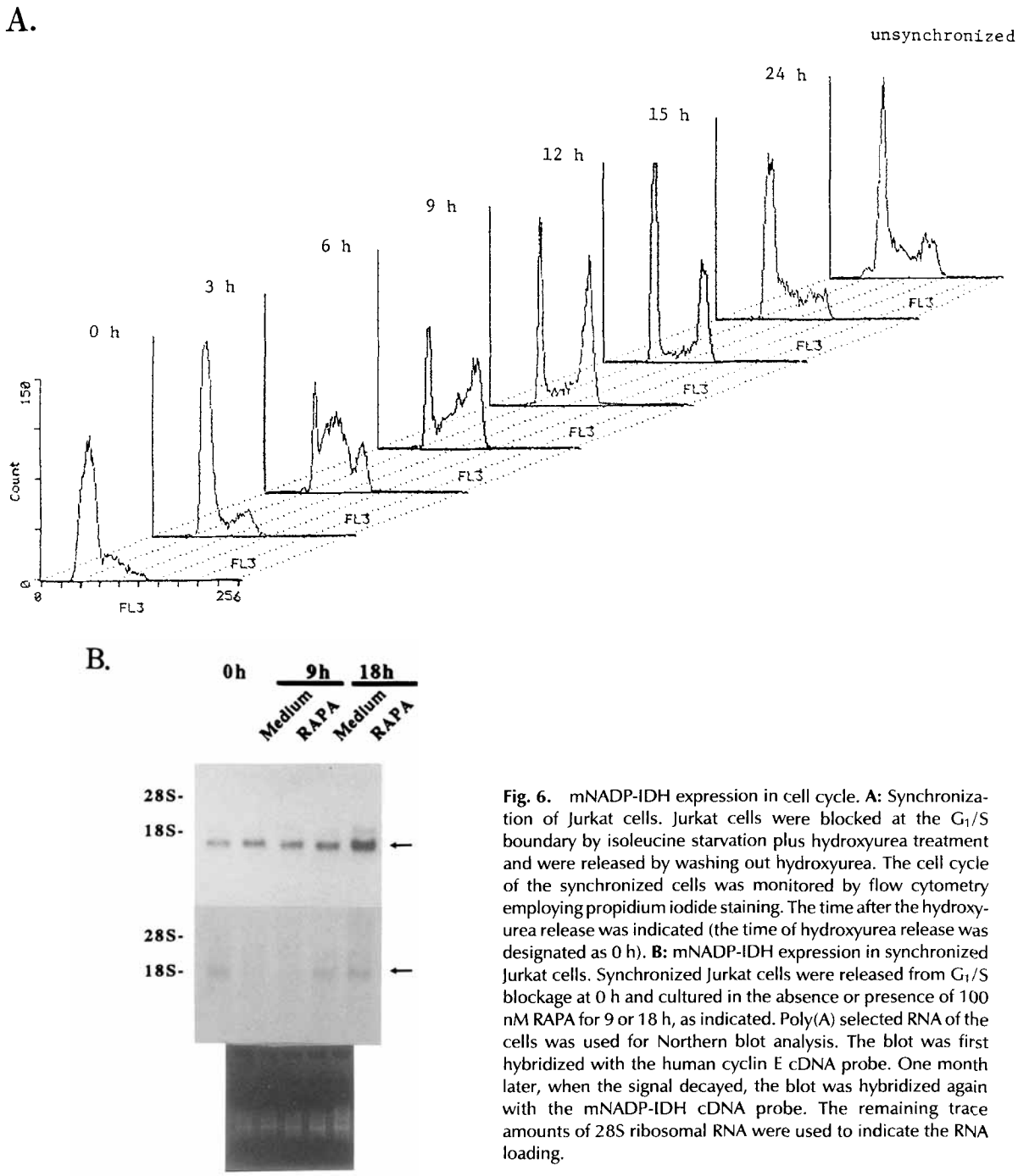

Fig. 6. mNADP-IDH expression in cell cycle. **A:** Synchronization of Jurkat cells. Jurkat cells were blocked at the G_1/S boundary by isoleucine starvation plus hydroxyurea treatment and were released by washing out hydroxyurea. The cell cycle of the synchronized cells was monitored by flow cytometry employing propidium iodide staining. The time after the hydroxyurea release was indicated (the time of hydroxyurea release was designated as 0 h). **B:** mNADP-IDH expression in synchronized Jurkat cells. Synchronized Jurkat cells were released from G_1/S blockage at 0 h and cultured in the absence or presence of 100 nM RAPA for 9 or 18 h, as indicated. Poly(A) selected RNA of the cells was used for Northern blot analysis. The blot was first hybridized with the human cyclin E cDNA probe. One month later, when the signal decayed, the blot was hybridized again with the mNADP-IDH cDNA probe. The remaining trace amounts of 28S ribosomal RNA were used to indicate the RNA loading.

Kreb's cycle, the relative contribution of NADP-dependent IDH to the anabolic process is less well understood. The cytosolic and mitochondrial NADP-IDHs catalyze the conversion between isocitrate and α-ketoglutarate and generate NADPH in the process. The two enzymes are also a part of the isocitrate shuttle, the net effect of which is to divert NADPH from the mitochondrion to cytosol. NADPH is an essential element for biosynthesis of lipids, amino acids, and DNA, while α-ketoglutarate is the critical precursor of glutamate. Considering the highly conserved nature of mNADP-IDH in polypeptide and cDNA sequences among different species, and that only a limited number of tissues (i.e., heart, muscle, and activated lymphocytes) had high levels of mNADP-IDH expression, the enzyme might play an important role for the function of these tissues. However, its role is probably not related to cell proliferation, because the gene expression in

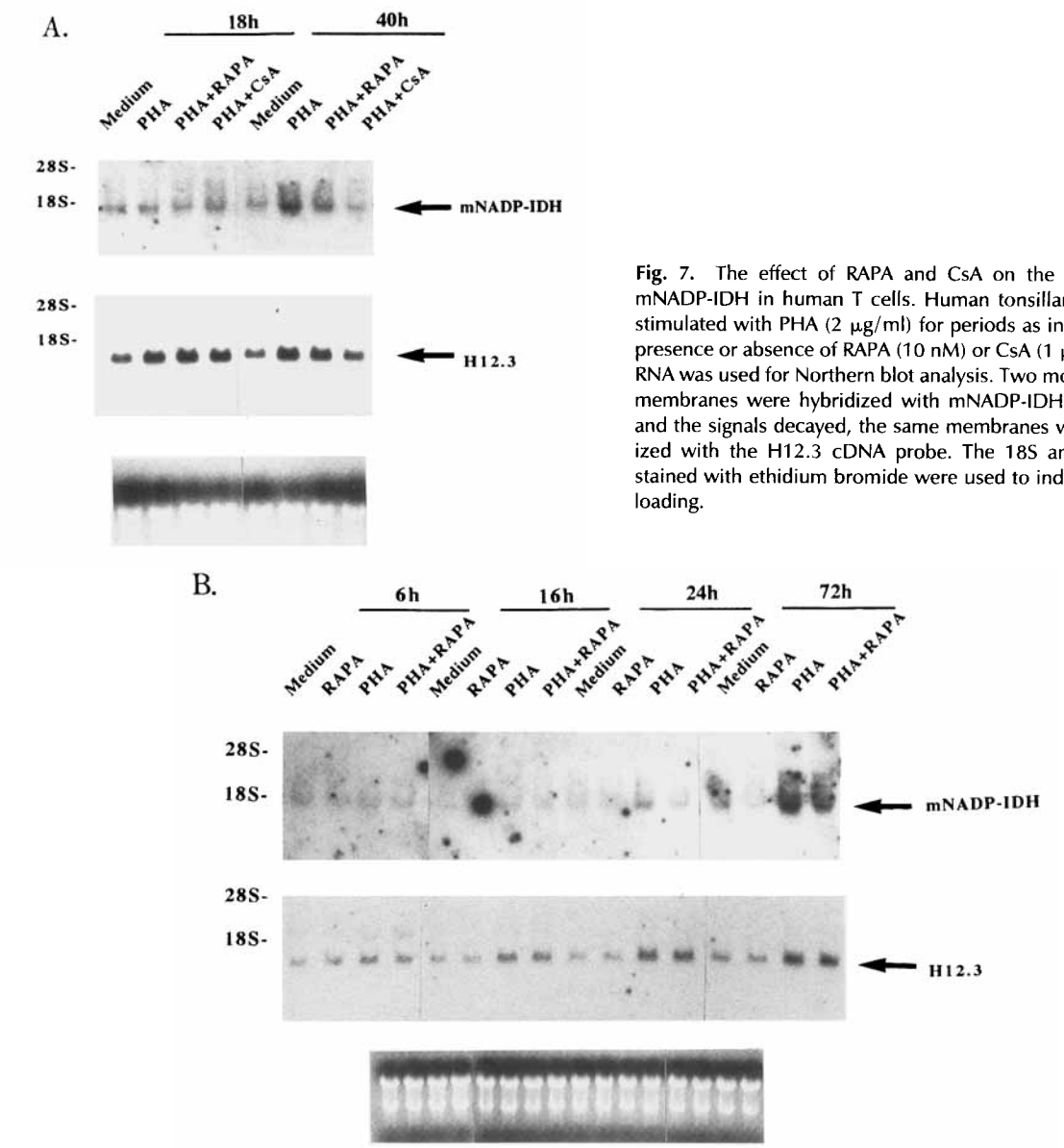

Fig. 7. The effect of RAPA and CsA on the expression of mNADP-IDH in human T cells. Human tonsillar T cells were stimulated with PHA (2 μg/ml) for periods as indicated in the presence or absence of RAPA (10 nM) or CsA (1 μM). The total RNA was used for Northern blot analysis. Two months after the membranes were hybridized with mNADP-IDH cDNA probe and the signals decayed, the same membranes were rehybridized with the H12.3 cDNA probe. The 18S and 28S bands stained with ethidium bromide were used to indicate the RNA loading.

nonproliferating cardiac cells was very high, while some fast proliferating cells such as HeLa and RVH had little expression of the gene. Further, although IL-2 and IL-4 had growth-promoting activities for activated T cells and IFN-γ and IL-6 for activated B cells [Okada et al., 1979; Wagner et al., 1978; Paul 1991; Hirano et al., 1987], these lymphokines did not augment the mNADP-IDH expression. This suggests that in lymphocytes the role of this enzyme is more related to the activation process.

In lymphocytes, it has been reported that the NADP/NADPH ratio drops from 1.4 in the resting cells to 0.2 in the cells 4 days after mitogen stimulation [Berger et al., 1982]. The hexose monophosphate shunt is known to be an important source of NADPH in lymphocytes, and the activity of this shunt increases during mitogen stimulation [Sagone et al., 1974]. It has been shown that in lymphocytes NADP-IDHs (i.e., cytosolic or mitochondrial) are the only other dehydrogenases which are involved in NADP reduction [Klein et al., 1990]. Our results indicate that there is a significant increase of steady-state mRNA and enzymatic activity of mNADP-IDH during lymphocyte activation. This increase is probably necessary to meet the augmented requirement for NADPH during cell activation. On the contrary, the cytosolic NADP-IDH remained below the detectable level in both the resting and activated lymphocytes. The differential expression of the cytosolic and mitochon-

drial NADP-IDH after the mitogen stimulation is an interesting new finding, and its physiological significance is yet to be explored.

The effect of a strong immunosuppressant, rapamycin, on mNADP-IDH expression in lymphocytes was examined in this study. RAPA is structurally related to FK506, which is also a strong immunosuppressant [Morris, 1992]. The mechanism of RAPA's effect is not clear. We only know that RAPA binds to an intracellular receptor called FK506 binding protein (FKBP), and the RAPA-FKBP complex inhibits the activation of p70 S6 ribosomal kinase [Kuo et al., 1991; Chung et al., 1992]. Whether such inhibition is responsible for the major drug effect is questionable, because recent study has shown that in an erythroid cell line MEL, the p70 S6 kinase activity can be totally inhibited by RAPA while the cells still proliferate vigorously [Calvo et al., 1994]. Several proteins terms TOR (target of RAPA) have been identified as they could bind to the RAPA-FKBP complex, yet the function of these TORs is to be further studied [Heitman et al., 1993; Kunz et al., 1993; Sabatini et al., 1994; Brown et al., 1994]. Terada et al. [1994] has reported that RAPA selectively inhibits translation of mRNAs encoding elongation factors and ribosomal proteins. Nevertheless, it is intriguing that RAPA does not seem to generally inhibit protein synthesis. A recent discovery is that RAPA prevents an inhibitory protein, $p27^{kip1}$, from disassociating from the cyclin E/Cdk2 complex during IL-2–induced T cell proliferation and thus prevents the cells from leaving the quiescent status [Nourse et al., 1994]. Whether this $p27^{Kip1}$-mediated mechanism can be generalized is to be investigated. Whatever the proposed mechanisms are, it remains puzzling that a strong immunosuppressant like RAPA has little effect on the expression of most early and late phase activation genes. To date, we have cloned or tested over 50 such genes (data not shown), and most of them, including H12.3 and cyclin E as shown in this study, were not sensitive to RAPA. Human mNADP-IDH is one of a few genes which is repressed by RAPA. It is to be noted that RAPA did not inhibit the mNADP-IDH expression in Jurkat cells as shown in this study, although the proliferation of these cells was repressed by RAPA. In a separate study, we have also noticed that the mitogen-induced mNADP-IDH expression in mouse spleen cells was not sensitive to RAPA, although the proliferation of the spleen cells was inhibited by RAPA (data not shown). Taken together, these data suggest that there might exist other critical target(s) of RAPA. In any event, this RAPA-sensitive human mNADP-IDH gene could be used to trace the upstream events affected by RAPA.

In spite of the fact that this study was carried out from an immunological perspective, some findings might be of interest to other disciplines. For example, the high level expression of the gene in the heart and muscle could be used as specific markers for the integrity of these tissues. Increased serum levels of NADP-IDH activity might signify the damage of these tissues due to various pathological conditions.

ACKNOWLEDGMENTS

The authors thank Mr. Liangpeng Yang for participating in cDNA sequencing. The authors also thank Dr. Suren Sehgal for providing rapamycin and Dr. Ted Bradley for providing RNA of malignant cell lines. The excellent secretarial assistance of Ms. Carmen Rodriguez is fully appreciated. This work was supported by grant MT-11543 from the Medical Research Council of Canada and a grant from the Kidney Foundation of Canada to J.W. and by the Nephrology Service of the Notre-Dame Hospital. J. Wu is a scholar of Fonds de la Recherche en Santé du Québec.

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