Interleukin-2 induces \( \gamma \)-S-adenosyl-L-methionine synthetase gene expression during T-lymphocyte activation

Rafael TOBENA*†, Saburo HORIKAWA‡, Victor CALVO* and Susana ALEMANY**‡

*Instituto de Investigaciones Biomedicas, CSIC, Fac. Medicina, IAM, Arturo Dupuyer 4, Madrid-28029, Spain, and †Department of Pathological Biochemistry, Medical Research Institute, Tokyo Medical and Dental University, Kanda-surugadai, Chiyoda-ku, Tokyo 101, Japan

The regulation of expression of the \( \gamma \)-S-adenosyl-L-methionine (AdoMet) synthetase gene was investigated in T-cells during \( G_0/G_1 \) transition, as well as throughout the \( G_1 \) phase. Stimulation of \( G_0 \) T-lymphocytes with concanavalin A induces AdoMet synthetase gene expression, starting 8 h after stimulation. Interleukin-2 (IL-2) stimulates the induction of this gene expression and AdoMet synthetase activity in \( G_0 \) lymphoblasts, in part by an increase in the transcription rate of the gene. Phorbol esters, which also stimulate the proliferation of \( G_1 \) lymphoblasts, show a similar kinetics of AdoMet synthetase mRNA induction. In contrast, the mRNA levels of the \( \gamma \)-adenosyl-L-homocysteine hydrolase, another enzyme of the methionine cycle, remain unchanged upon IL-2 or phorbol 12,13-dibutyrate treatment. Dexamethasone and 8Br-cAMP, both inhibitors of lymphocyte proliferation, are able to block the expression of the AdoMet synthetase gene and, consequently, AdoMet synthetase activity. Together these findings indicate that the AdoMet synthetase gene is subject to cell-cycle regulation in T-lymphocytes.

INTRODUCTION

S-Adenosyl-L-methionine (AdoMet) synthetase catalyses the formation of AdoMet from methionine and ATP [1]. The AdoMet synthetase gene is included in the 482 near-minimal set of genes necessary for independent life [2], indicating that the product of this reaction is essential for life. AdoMet is the methyl donor in almost all transmethylation reactions [1]. A product of these reactions is S-adenosyl-L-homocysteine (AdoHcy). The hydrolysis of AdoHcy by AdoHcy hydrolase (EC 3.3.1.1), a reversible enzyme, generates adenosine and homocysteine [3].

In mammalian tissues, three distinct forms of AdoMet synthetase have been identified and designated as AdoMet synthetases \( \alpha \), \( \beta \) and \( \gamma \), and all these forms have different biochemical properties [4,5]. On the other hand, the \( \gamma \) form is widely distributed in various tissues examined [4,6]. The \( \gamma \) form in human lymphocytes is composed of three polypeptide chains of 53, 51 and 38 kDa [7]. The 51 kDa protein appeared to be derived from the 53 kDa protein [7]. We have cloned the catalytic 53/51 kDa protein subunit cDNAs from rat [6] and human [8] kidney cDNA libraries, and a human lymphocyte cDNA has also been cloned recently [9].

Lymphocytes isolated from spleen or lymph nodes are in \( G_0 \) phase of the cell cycle [10]. Stimulation with the antigen-presenting cell or with mitogenic lectins, like concanavalin A (Con A), activates at least two signalling pathways involving an elevation of cytosolic \( Ca^{2+} \) and diacylglycerol levels [11]. As a consequence, quiescent T-lymphocytes enter the \( G_1 \) phase of the cell cycle, where they produce interleukin-2 (IL-2), and high-affinity receptors for this interleukin appear on the plasma membrane [12]. Transition from \( G_1 \) to \( S \) phase occurs as a result of IL-2 binding to its high-affinity receptor [13]. Phorbol esters, are also able to induce \( G_1 \) lymphoblast entry into the \( S \) phase by a different intracellular signalling pathway [14,15].

In this paper we demonstrate that the expression of the \( \gamma \) AdoMet synthetase gene and AdoMet synthetase activity are induced during T-lymphocyte activation as a consequence of the interaction of IL-2 with its high-affinity receptors, while AdoHcy hydrolase mRNA levels remain unchanged. The increase in AdoMet synthetase gene expression is at least in part due to an increase in the transcription rate of the gene. Phorbol esters also stimulate \( \gamma \) AdoMet synthetase gene expression and activity in \( G_0 \) lymphoblasts. Finally, the effects of inhibitors of lymphocyte activation on the expression of this gene have also been studied.

MATERIALS AND METHODS

Isolation of quiescent lymphocytes

Rat lymph nodes were aseptically removed and placed into RPMI medium (Gibco) with 60 mg/ml gentamicin (Llorente) and 0.25 mg/ml fungizone (Sigma). Lymphocytes were obtained as in [14]. Briefly, filtered cells were pelleted, resuspended for 2 min in buffered 0.14 M ammonium chloride, pH 7.4, and washed twice with RPMI supplemented with gentamicin, fungizone and 2% (v/v) heat-inactivated fetal calf serum (Gibco). To purify T-lymphocytes, nylon wool (Du Pont-New-England Nuclear) columns were used as described in [14]. Lymphocytes were incubated at 37 °C in humidified 95% air/5% \( \text{CO}_2 \) for 1 h and the non-adherent cells were used for stimulation.

Obtention of lymphoblasts and cell proliferation

Lymphoblasts were obtained by stimulation of spleen lymphocytes at a concentration of \( (5-7) \times 10^6 \) cells/ml for 48 h with 2 \( \mu \)g/ml Con A (Aldrich) [16,17]. Cell proliferation assays were performed as described in [14].

Northern blot analysis

Quiescent lymphocytes (\( 1 \times 10^6 \)) were incubated with 2 \( \mu \)g/ml Con A, 50 ng/ml phorbol 12,13-dibutyrate (PDBu; Sigma) or...
0.25 µM ionophore A 23187 (Boehringer Mannheim). Lymphoblasts (2 × 10^5) were incubated with human recombinant IL-2 (20 units/ml; Hoffmann-La Roche Inc.), 1 × 10^{-7} M dexamethasone (DEX; Sigma) and/or 0.4 mM 8Br-cAMP (Boehringer Mannheim). After stimulation, cells were grown at 37°C in humidified 5% air/5% CO₂, and at different times cells were washed with cold PBS and pelleted. Total cellular RNA was isolated from cells by extraction in guanidinium thiocyanate as described elsewhere [18]. Total RNA (20 µg) was electrophoresed through a 2.2 M formaldehyde/1% agarose gel in 1× Mops buffer (pH 7) and blotted onto a nylon membrane (Nytran, NY 13N; Renner GmbH). Filters were hybridized with either a 1.2 kb fragment of a rat γ AdoMet synthetase cDNA clone [6] or with a 1.9 kb fragment of a rat AdoHcy hydrolase cDNA clone [3], labelled using the random primer technique (10^6 c.p.m./µg). The rat γ AdoMet synthetase cDNA fragment does not cross-hybridize with the other described synthetase mRNA species (α/β), which are expressed only in hepatic tissue [4,5]. Blots were hybridized for 16 h at 42°C in the presence of 50% formamide/5× SSC (0.15 M NaCl/0.015 M sodium citrate)/50 mM sodium phosphate (pH 6.5)/5× Denhardt’s solution (0.02% Ficoll 400/0.02% polyvinylypyrrolidone/0.002% BSA), washed twice at room temperature in the presence of 2× SSC/0.1% SDS for 15 min, twice at 65°C in the presence of 2× SSC/0.1% SDS for 15 min, twice at 65°C in the presence of 1× SSC/0.1% SDS for 30 min and, finally, two washes were performed at 65°C in the presence of 0.3× SSC/0.1% SDS for 30 min. Membranes were exposed to X-ray film for 36 h at −70°C.

**Measurement of AdoMet synthetase activity**

Cells were stimulated with IL-2 or PDBu, as described above. At different times, 20 × 10^3 cells were pelleted, washed with PBS and frozen at −20°C. Cells were homogenized by freezing and thawing three times in 10 mM Tris/HCl, pH 7.5, 0.3 M sucrose, 0.1 mM EGTA, 1 mM benzamidine and 0.1% 2-mercaptoethanol and centrifuged in a microfuge at maximal speed for 15 min. AdoMet synthetase activity was measured, in the presence of 5 mM methionine, in the supernatant as described in [19].

**Nuclear run-on transcription assay**

Control or IL-2-stimulated lymphoblasts (2 × 10^5; 5.5 h) were washed twice with cold PBS and lysed with 1 ml of lysis buffer [10 mM Tris/HCl (pH 7.4)/10 mM NaCl/3 mM MgCl₂/0.5% Nonidet P40/50 units/ml RNase inhibitor (Boehringer Mannheim)], incubated for 3 min on ice and then centrifuged at 1000 g for 3 min. Nuclei were washed once with reaction buffer [20 mM Tris/HCl (pH 8.0)/2.5 mM MgCl₂/150 mM KCl/1 mM dithiothreitol/20% (v/v) glycerol], resuspended in 100 µl of storage buffer (2× reaction buffer plus 500 units/ml RNase inhibitor) and frozen in liquid nitrogen. For the *in vitro* transcription reaction, nuclei were thawed on ice and incubated with 0.5 mM ATP, CTP, GTP (Boehringer Mannheim), 2.4 mM creatine phosphate (Sigma), 0.02 mg/ml creatine kinase (Boehringer Mannheim), 500 units/ml RNase inhibitor and 150 µCi of [³²P]UTP (3000 Ci/mmol) (NEG. 007H, New England Nuclear) in a total volume of 200 µl, for 45 min at 26°C. Then, 5 µl of 50 mM CaCl₂ and 25 µl of 10 units/ml DNase (Boehringer Mannheim) were added and incubated at 26°C for another 30 min. The digest was incubated with 10 µl of 10 µg/ml proteinase K in 600 µl of solution containing 200 mM Tris/HCl (pH 7.4), 50 mM EDTA and 2% SDS for 30 min at 42°C. A 10 µl aliquot of 10 mg/ml yeast tRNA (Boehringer Mannheim) and 80 µl of 2 M sodium acetate, pH 5.2, were added, and nuclear RNA was extracted with 800 µl of water-saturated phenol and 200 µl of chloroform/isoamyl alcohol (24:1). The aqueous phase was precipitated with cold ethanol and the pellet was washed with 75% ethanol. The pellet was then resuspended in 50 µl of 0.2 M NaOH, incubated on ice for 10 min and then neutralized to pH 7.4 with 20 µl of 2.4 M Hepes free-acid. The solution was applied to a Nick Column (Pharmacia LKB Biotechnology Inc., Uppsala, Sweden) that had previously equilibrated with RNase-free water. The radioactivity of the RNAs transcribed *in vitro* was determined, and equal amounts of radioactive RNA from IL-2-stimulated or unstimulated cells were used to hybridize with 5 µg of the plasmids: AdoHcy hydrolase-pBluescript, γ AdoMet synthetase-pUC118, glycerol-dehyde-phosphate dehydrogenase (GADPH)-pGEM3Z, pBluescript, pUC118 and pGEM3Z, previously applied to a nylon matrix (Nytran NY 13N; Renner GmbH) with the aid of a slot blot apparatus. The nylon membranes containing the plasmids were prehybridized for 4 h in prehybridization buffer containing 1 mg/ml yeast tRNA at 65°C, followed by hybridization with the [³²P]-labelled RNA for 72 h at 65°C. The filters were washed twice with 2× SSC for 30 min at 65°C, once with 2.5 mg/ml of RNase A and 5 units/ml of RNase T1 in 2× SSC for 30 min at 37°C, and once with 2× SSC at 25°C for 15 min, and then exposed to X-ray film for 3 days and quantified by computer-assisted densitometry.

**RESULTS**

To determine whether γ AdoMet synthetase gene expression could be induced in resting T-lymphocytes in response to Con A, a potent inducer of T-cell proliferation, we isolated total RNA at different times after Con A-stimulation of lymph node T-cells, and Northern blots were performed. When resting lymphocytes were stimulated with this lectin, γ AdoMet synthetase mRNA levels started to increase as early as 8 h after stimulation, reached maximum 16 h after stimulation, and stayed above basal levels as late as 24 h after stimulation (Figure 1A). Activation of G₁-T-lymphocytes by Con A leads to a simultaneous increase in both intracellular Ca²⁺ and diacylglycerol. The possible involvement of these intracellular messengers in AdoMet synthetase induction was tested separately. As shown in Figure 1(C), treatment of these cells with either PDBu, a phorbol ester that mimics diacylglycerol by activating protein kinase C (PKC) or Ca²⁺

![Figure 1: Induction of γ AdoMet synthetase gene expression by Con A or the Ca²⁺ ionophore A23187 and phorbol esters in T-lymphocytes](image-url)
 Ionophore A23187 separately, did not induce expression of this gene. However, both signals, Ca\textsuperscript{2+} increase and PKC stimulation, were necessary to elicit a response (Figure 1C). Activation of both signal-transduction pathways is needed for the appearance of high-affinity IL-2 receptors on the T-cell plasma membrane and for IL-2 secretion. About 5 h after Con A stimulation, lymphocytes start to secrete IL-2 to the extracellular medium, and IL-2 interacts with its high-affinity receptors [13]. All these data suggest that IL-2 could regulate \(\gamma\)AdoMet synthetase mRNA levels.

To test this hypothesis, G\textsubscript{1} phase lymphoblasts were stimulated with recombinant IL-2, and Northern blots were performed. Addition of IL-2 to lymphoblasts obtained from rat spleen induced an increase in the steady-state levels of \(\gamma\)AdoMet synthetase mRNA. The maximal increase, 5.3 \(\pm\) 0.5-fold (\(n = 3\)), in \(\gamma\) AdoMet synthetase mRNA levels could be detected 6 h after IL-2 stimulation (Figure 2A), but \(\gamma\) AdoMet synthetase mRNA levels remained increased as late as 20 h after IL-2 stimulation, when lymphoblasts were entering the S phase of the cell cycle, as monitored by incorporation of radiolabelled thymidine into the cells (results not shown). IL-2 stimulation of lymphoblasts did not induce an increase in AdoHcy hydrolase mRNA levels (Figure 2B).

Phorbol esters, like IL-2, are able to stimulate lymphoblast proliferation. However, different signalling pathways have been proposed for this proliferative effect [14,15]: IL-2 exerts its action through a PKC-independent mechanism and phorbol esters operate through PKC activation. Addition of PDBu (50 ng/ml) induced an increase in \(\gamma\) AdoMet synthetase mRNA levels. The time course of \(\gamma\) AdoMet synthetase gene expression was quite similar to that observed with IL-2 (Figure 2D). The increase in mRNA AdoMet synthetase levels after 6 h of PDBu stimulation, compared with unstimulated levels, has been calculated to be about 5.8 \(\pm\) 0.4-fold (\(n = 3\)). AdoHcy hydrolase mRNA levels remained unchanged after PDBu activation of lymphoblasts (Figure 2E).

To determine whether IL-2 or PDBu induction of \(\gamma\) AdoMet synthetase mRNA resulted in a concomitant increase in AdoMet synthetase activity, this enzyme activity was measured at different times after lymphoblast stimulation. As shown in Figure 3, an increase of about 4-fold in AdoMet synthetase activity was observed after IL-2 or PDBu stimulation of lymphoblasts. The highest value for AdoMet synthetase activity was observed about 8 h after lymphoblast stimulation, 2 h later than the observation of maximal levels of \(\gamma\) AdoMet synthetase mRNA. Maximal AdoMet synthetase activity was still observed 12 h after PDBu or IL-2 stimulation, when maximal levels of \(\gamma\) AdoMet synthetase mRNA levels were already decreasing.

An increase in the level of mRNA can be the result of increases in the transcription rate of the gene and/or the half-life of the messenger. Nuclear run-on assays were performed to determine whether the effects of IL-2 on AdoMet synthetase expression in T-lymphoblasts were related to increases in the rate of transcription of this gene. Treatment of T-lymphoblasts with IL-2 was associated with a 3-fold increase in the rate of AdoMet
The mRNA levels and the transcription rate of another enzyme, GADPH gene (Figure 4). The transcription run-on assay was performed with nuclei isolated from lymphocytes treated or not for 5.5 h with IL-2. (A) Nylon matrices containing the indicated plasmids were hybridized with the ‘in vitro’ transcribed RNAs. (B) The histogram shows the quantification of GADPH, AdoHcy hydrolase and AdoMet synthetase transcription rates normalized to the corresponding vectors. The transcription rates were calculated as the ratios of stimulated versus unstimulated values. This panel shows the mean of two different experiments. C, control.

**Figure 4** Nuclear run-on analysis of the AdoMet synthetase and AdoHcy hydrolase transcription rates after IL-2 treatment of rat lymphoblasts

The transcription run-on assay was performed with nuclei isolated from lymphocytes treated or not for 5.5 h with IL-2. (A) Nylon matrices containing the indicated plasmids were hybridized with the ‘in vitro’ transcribed RNAs. (B) The histogram shows the quantification of GADPH, AdoHcy hydrolase and AdoMet synthetase transcription rates normalized to the corresponding vectors. The transcription rates were calculated as the ratios of stimulated versus unstimulated values. This panel shows the mean of two different experiments. C, control.

DISCUSSION

This study demonstrates that IL-2 or PDBu treatment of G1 phase T-lymphocytes results in up-regulation of γ AdoMet synthetase gene expression. The good correlation, both in the kinetics and in the increases in γ AdoMet synthetase mRNA levels (5.3-fold) and AdoMet synthetase activity (4-fold), suggests that the increase in activity is due, at least in part, to an increase in the mRNA levels. Nuclear run-on assays further demonstrated that the induction of γ AdoMet synthetase expression by IL-2 in T-lymphoblasts is at least in part controlled by a transcriptional mechanism. The half-life of the γ AdoMet synthetase transcript in IL-2- or PDBu-stimulated lymphoblasts is identical (about 4 h; results not shown), indicating that PDBu probably also controls, as IL-2, the mRNA levels of γ AdoMet synthetase, at least partially, through a transcriptional mechanism.

The mRNA levels and the transcription rate of another enzyme of the methionine cycle, AdoHcy hydrolase, remain unchanged after IL-2 stimulation. These observations indicate that AdoMet

Table 1 Comparison of AdoMet synthetase mRNA levels, AdoMet synthetase activity and cell proliferation in IL-2- and PDBu-stimulated lymphoblasts preincubated with DEX or cAMP

<table>
<thead>
<tr>
<th></th>
<th>+ IL-2</th>
<th>+ PDBu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cAMP</td>
<td>DEX</td>
</tr>
<tr>
<td>Lymphoblast proliferation</td>
<td>44 ± 5</td>
<td>2 ± 0.5</td>
</tr>
<tr>
<td>AdoMet synthetase activity (%)</td>
<td>40 ± 3</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>AdoMet synthetase mRNA levels (%)</td>
<td>32 ± 5</td>
<td>9 ± 1</td>
</tr>
</tbody>
</table>

Cells preincubated for 30 min with 8Br-cAMP (0.4 mM) or DEX (10⁻⁷ M) and stimulated with IL-2 or PDBu for 6 h were harvested and total RNA was isolated. (A) Northern blot analysis of lymphoblasts stimulated with IL-2 (20 units/ml) in the presence or absence of 8Br-cAMP or DEX. (B) Methylene Blue staining of the membrane after transfer corresponding to (A) (upper) and (C) (lower). (C) Northern blot analysis of lymphoblasts stimulated with PDBu (50 ng/ml) in the presence or absence of 8Br-cAMP or DEX.

**Figure 5** Regulation of AdoMet synthetase gene expression by 8Br-cAMP and DEX in G1 lymphoblasts

Cells preincubated for 30 min with 8Br-cAMP (0.4 mM) or DEX (10⁻⁷ M) and stimulated with IL-2 or PDBu for 6 h were harvested and total RNA was isolated. (A) Northern blot analysis of lymphoblasts stimulated with IL-2 (20 units/ml) in the presence or absence of 8Br-cAMP or DEX. (B) Methylene Blue staining of the membrane after transfer corresponding to (A) (upper) and (C) (lower). (C) Northern blot analysis of lymphoblasts stimulated with PDBu (50 ng/ml) in the presence or absence of 8Br-cAMP or DEX.
synthetase could be one of the regulatory steps in the methionine cycle.

8Br-cAMP and DEX have been described as inhibitors of lymphocyte activation [20–23]. Little is known about the molecular events responsible for these alterations. Some genes are induced as a consequence of DEX treatment in lymphocytes, but expression of others is repressed [14,22–24]. The data shown in Figure 5 and Table 1 indicate that γ AdoMet synthetase mRNA levels and activity are regulated by the two different immuno-suppressants. We, and others, have shown that Raf-1 kinase is necessary for IL-2- or phorbol ester-induced lymphoblast proliferation [25,26]. Interestingly, cAMP inhibits Raf-1 kinase activation [27]. On the other hand, activation of p70/S6 kinase in IL-2-responsive lymphoid cells is inhibited by cAMP [28]. Further experiments have to be carried out to determine if Raf-1 and/or p70/S6 kinases control γ AdoMet synthetase gene expression in rat lymphoblasts.

This report is the first evidence that γ AdoMet synthetase gene expression is controlled during the cell cycle. The α/β AdoMet synthetase gene is selectively expressed only in liver [4,5], and, to our knowledge, no regulation of the expression of the α/β AdoMet synthetase gene has been described during the cell cycle. In fact, α/β AdoMet synthetase mRNA could not be detected in G1 or G2 phase T-lymphocytes after Con A or IL-2 stimulation. (M. A. Pajares, L. Alvarez and R. Toñé, personal communication). Our data indicate that the expression of the two genes is differently regulated and that the expression of the γ AdoMet synthetase gene can be controlled by a proliferative signal.

AdoMet is the methyl donor in almost all transmethylation reactions [1]. A product of these reactions, AdoHcy, is hydrolysed by AdoHcy hydrolase, generating adenosine and homocysteine [3]. AdoMet is also a precursor of polyamines [29].

It has been shown that in proliferating T-lymphoblasts, levels of AdoMet, AdoMet utilization and homocysteine are increased compared with G1 lymphocytes [30]. However, our results demonstrate that the enzymes responsible for the generation of AdoMet and the conversion of AdoHcy into homocysteine exhibit differential regulation in IL-2-stimulated T-lymphoblasts: γ AdoMet synthetase transcription is increased while AdoHcy hydrolase transcription remains unchanged. AdoHcy hydrolase is a reversible enzyme [3] and is not a rate-limiting step in the methionine cycle. Thus, an increase in AdoHcy levels due to AdoMet utilization in transmethylation reactions could automatically result in an increase in homocysteine levels. However, post-translational modifications that could activate AdoHcy hydrolase cannot be excluded.

An increase in the levels of polyamines has been described to be essential for proliferation and differentiation in many cell systems [29,31], including T-lymphocytes [32]. This increase in the synthesis of polyamines requires a higher production of AdoMet. In phytohaemagglutinin-stimulated lymphocytes the levels of AdoMet are increased compared with G1 lymphocytes [30]. The level of transcripts for the key enzymes in polyamines biosynthesis, AdoMet decarboxylase and ornithine decarboxylase, are also increased after activation of lymphocytes with mitogenic lectins [33,34]. Based on the results presented here, we conclude that IL-2-triggered lymphocyte activation increases AdoMet synthetase as a consequence of the increase in γ AdoMet synthetase transcript level. It would interesting to know whether the regulation of γ AdoMet synthetase gene expression by a mitogenic signal is T-cell specific or is a general event, necessary for the increase in polyamine levels during G1 phase of the cell cycle.

We are grateful to M. A. Pajares and L. Alvarez for critical discussion of the manuscript and to J. M. Mato’s laboratory for the facilities provided. We also thank Drs. Cantoni and Akssmit for providing the AdoHcy hydrolase CDNA probe, and Dr. M. K. Gately (Hoffmann-La Roche Inc.) for the recombinant IL-2. R.T. is the recipient of a fellowship from Plan Nacional. This work was supported by grants from Plan Nacional, Comunidad de Madrid and Europharma.

Received 8 May 1996/19 June 1996; accepted 8 July 1996

REFERENCES


Received 8 May 1996/19 June 1996; accepted 8 July 1996