Induction of Interleukin 2 Production but not Methionine Adenosyltransferase Activity or S-Adenosylmethionine Turnover in Jurkat T-Cells

James De La Rosa, Arthur M. Geller, H. Leighton LeGros, Jr., and Malak Kotb


ABSTRACT

We have recently reported that methionine adenosyltransferase (MAT) in resting human peripheral blood T-cells is primarily present in the form of a precursor which we named A. This protein decreases upon cell stimulation, as both MAT activity and the amount of the catalytic a/α' subunits of the enzyme increase. When resting cells are activated by phytohemagglutinin, the decrease in λ and increase in α/α' occurs after interleukin 2 (IL-2) production and before DNA synthesis. The human T-lymphoma cell line, Jurkat, is unique in its ability to produce IL-2 in response to exogenous stimuli such as T-cell mitogens and therefore provides a convenient model for studying biochemical reactions involved in T-cell activation. In this study the regulation of MAT activity and S-adenosylmethionine (AdoMet) in resting and activated Jurkat cells was investigated. Here we report that MAT activity in unstimulated Jurkat cells is about 10- and 3-fold higher than the activity in resting and activated peripheral blood mononuclear cells, respectively. Activation of Jurkat cells with phytohemagglutinin resulted in increased IL-2 production, but not an increase in MAT activity. Identical results were obtained using freshly isolated cells from acute lymphoblastic leukemia patients. AdoMet utilization and pool size were approximately 3- and 10-fold higher, respectively, in Jurkat cells compared to peripheral blood mononuclear cells, and both parameters were unaffected by phytohemagglutinin stimulation. Jurkat MAT was determined to be structurally indistinguishable from enzyme from T- or B-leukemia cells but was different from resting, normal T-cells in that it lacked the λ form. Furthermore, unlike MAT in resting T-cells, the relative amounts of the α, α', and β subunits of the enzyme did not change throughout the course of IL-2 induction. We conclude that AdoMet metabolism and MAT activity in Jurkat cells are constitutively high and that induction of IL-2 synthesis in these cells is independent of changes in AdoMet synthesis or turnover. The lack of the λ form and the difference in MAT regulation between leukemic T-cells and peripheral blood mononuclear cells may be exploited in the design of specific chemotherapeutic agents.

INTRODUCTION

AdoMet1 donates methyl groups to proteins, nucleic acids, and various lipids and donates propylamine groups for the biosynthesis of polyamines. The importance of AdoMet in proliferating cells is underscored by the well established role polyamines play in proliferation, the recently discovered role of protein methylation in the processing of ras proteins (1, 2), and the reports that DNA methylation is important in the regulation of different lymphocyte genes upon cell activation and differentiation (4–6).

Considering the importance of AdoMet in lymphocytes, it is surprising that there is very little information regarding its rate of utilization during the course of cell activation. One exception is the report by German et al. (7) which documented that after stimulation of PBMC with PHA, the AdoMet pool size and rate of AdoMet utilization increased (7). Subsequent studies by Kotb et al. (8) and De La Rosa et al. (9, 10) showed that upon activation of PBMC, MAT activity also increased. More recently, we discovered that in resting human T-cells, MAT is present in a precursor form called λ (10). Upon lymphocyte activation λ, which has low MAT activity, disappears and the α/α' subunits, which have high MAT activity, increase concomitantly. The replacement of λ by α/α' subunits occurs after IL-2 synthesis and before DNA synthesis (10).

The human leukemic T-cell line, Jurkat, provided cell biologists with an excellent tool to study events of T-cell activation in a homogeneous population of cells because of its unique ability to produce IL-2 in response to mitogens and certain superantigens (11). We have used this T-cell line to study the regulation of MAT activity and AdoMet metabolism in lymphocytes. We report here that in Jurkat cells, unlike resting normal human T-cells, activation is not accompanied by either a change in the relative amounts of MAT subunits or an increase in MAT activity, AdoMet levels, or AdoMet turnover. AdoMet levels and MAT activity are constitutively higher in Jurkat cells compared to either resting or activated T-cells. The differential regulation of MAT in normal and leukemic T-cells may in part be due to the fact that Jurkat cells lack the λ form of the enzyme.

MATERIALS AND METHODS

Materials. Aprotinin, antipain, chymotrypsin, leupeptin, pepstatin A, soybean trypsin inhibitor, benzamidine, o-phenanthroline, phenylmethylsulfonyl fluoride, PHA, bicinchoninic acid, Ficoll-Hypaque, and the iodide salt of AdoMet were from Sigma Chemical Co. (St. Louis, MO). The tissue culture media and fetal bovine serum were from Gibco BRL (Gaithersburg, MD). L-[35S]Methionine was purchased from Du Pont New England Nuclear (Wilmington, DE), and the chemiluminescence detection system (ECL) was from Amersham (Arlington Heights, IL). Nitrocellulose membranes and horseradish peroxidase-conjugated goat anti-rabbit polyclonal antibody were from Bio-Rad (Richmond, CA) and XAR-5 film was from Kodak (Rochester, NY).

Leukemic T-Cells. Freshly isolated ALL-2 cells (T-cells, mithomyocye) from pediatric patients were provided by Dr. F. Behm at St. Jude Children's Research Hospital. The Jurkat cells were from the American Type Tissue Culture Collection (Rockville, MD). Cells were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mm L-glutamine, 50 μg/ml of streptomycin, and 50 units/ml of penicillin (referred to as RPMI complete). All cells were cultured at 37°C in humidified air containing 5% CO2.

Preparation of Cell Extracts. Cell extracts were prepared for analysis of AdoMet and t-methionine by lysing cells, precipitating protein with 2 N perchloric acid, and then neutralizing the extract with KOH and

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1 The abbreviation used is: AdoMet, S-adenosylmethionine; PBMC, peripheral blood mononuclear cells; MAT, methionine adenosyltransferase (ATP: methionine S-adenosyltransferase, EC 2.5.1.6); PHA, phytohemagglutinin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate buffered saline (140 mm NaCl, 2.7 mm KCl, 1.5 mm KH2PO4, and 8.1 mm Na2HPO4, pH 7.4); HPLC, high performance liquid chromatography; LDH, lactate dehydrogenase; IL-2, interleukin 2; PMA, phorbol myristic acetate.

2 The abbreviations used are: AdoMet, S-adenosylmethionine; PBMC, peripheral blood mononuclear cells; MAT, methionine adenosyltransferase (ATP: methionine S-adenosyltransferase, EC 2.5.1.6); PHA, phytohemagglutinin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PBS, phosphate buffered saline (140 mm NaCl, 2.7 mm KCl, 1.5 mm KH2PO4, and 8.1 mm Na2HPO4, pH 7.4); HPLC, high performance liquid chromatography; LDH, lactate dehydrogenase; IL-2, interleukin 2; PMA, phorbol myristic acetate.
MAT ACTIVITY AND AdoMet METABOLISM IN JURKAT CELLS

The specific activity of intracellular and extracellular L-methionine was determined after adding 2.5 to 3.0 μCi of L-[35S]methionine to snap top vials containing a culture of 10⁷ cells in 1 ml of RPMI complete. The vials were kept at 37°C water bath and at specific times, within 90 s, 0.8 ml of culture was removed and diluted into 50 ml of ice cold PBS. Both the vial and the conical centrifuge tube containing the diluted cells were then centrifuged for 1 min at 1800 × g. The medium in the vial was collected and used to determine the specific radioactivity of extracellular methionine. The cells in the conical tube were washed briefly with ice cold PBS, frozen in liquid nitrogen, and stored at −70°C until extracted (as described above) for intracellular methionine. Endogenous AdoMet was removed from the cell extract by adsorption to acid washed charcoal, which was added to the extract at 65 μg/10⁶ cells. Both extracellular and intracellular L-methionine in the extracts were converted enzymatically to AdoMet. To convert methionine to AdoMet, extracts were incubated with radiolabeled methionine at 3 nCi/ml for up to 2 h. At specific times 3 × 10⁶ cells were harvested, centrifuged for 1 min at 1800 × g, washed with PBS, and frozen as a cell pellet in liquid nitrogen until extracted. Cell extracts were made as described above, and the specific radioactivity of the AdoMet was determined by HPLC and liquid scintillation counting.

RESULTS

The Jurkat human T-cell line was studied for two reasons: (a) T-cells respond to PHA and are responsible for the changes observed in AdoMet metabolism and MAT activity in PHA-activated PBMC; (b) although Jurkat is a transformed cell line which proliferates continuously, it is considered a relatively resting T-cell line because, unlike other T-cell lines, it can be induced to secrete IL-2. Therefore, it was reasoned that because Jurkat cells can be activated, they might serve as a model to study the role of AdoMet metabolism in the induction of IL-2 synthesis. Induction of IL-2 Synthesis in Jurkat Cells Is Not Accompanied by an Increase in MAT Activity. Jurkat cells were incubated with different concentrations of PHA to determine the optimal concentration for cell activation, as indicated by IL-2 production, and for the induction of MAT activity. This dose response experiment demonstrated that IL-2 production by the Jurkat cells increased concomitantly with increasing PHA concentration, reaching a maximum at 2.5–5 μg/ml (Fig. 1A). However, MAT activity did not increase upon Jurkat activation, as was previously found for PBMC, and in fact appeared to decrease (Fig. 1B). LDH, which is a noninducible enzyme, was used as a negative control, and its activity remained relatively constant upon activation (Fig. 1C).

Freshly Isolated Leukemic T-Cells Behave Like Jurkat Cells. Next we asked whether the lack of induction of MAT activity represented an idiosyncratic behavior of the Jurkat clone or a general characteristic of leukemic T-cells. Freshly isolated PBMC from patients with ALL-2 were incubated for various times with optimal mitogenic concentrations of PMA plus ionomycin and then tested for IL-2 production and MAT activity. PMA and ionomycin were used instead of PHA be-

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MAT ACTIVITY AND AdoMet METABOLISM IN JURKAT CELLS

Fig. 1. PHA dose response of IL-2 production (A), MAT activity (B), and LDH activity (C). Jurkat cells at a density of 4.0 x 10^6 cells/ml were incubated with different concentrations of PHA and the cells were harvested after either 10 h (O) or 25 h (•). The media were assayed for IL-2 and the cell extracts were assayed for both MAT and LDH activity.

Table 1 Induction of IL-2 synthesis but not MAT activity in freshly isolated ALL-2 cells

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>IL-2 activity (units/ml)</th>
<th>MAT activity (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero time</td>
<td></td>
<td>11.3</td>
</tr>
<tr>
<td>24 (-)</td>
<td>3.6</td>
<td>10.3</td>
</tr>
<tr>
<td>24 (+)</td>
<td>118</td>
<td>12.6</td>
</tr>
</tbody>
</table>

AdoMet Utilization in Resting and Activated Jurkat Cells. Although the above experiments showed that MAT activity did not increase upon activation of Jurkat cells, this did not preclude the possibility that AdoMet utilization or pool size could change. To estimate the absolute rate of AdoMet utilization in stimulated and unstimulated Jurkat cells, the equilibration of extracellular radiolabeled methionine with intracellular methionine and AdoMet was determined. The time course of this equilibration is shown in Fig. 3. In both stimulated and unstimulated Jurkat cells methionine specific radioactivity reached half-maximum in about 15 s (Fig. 3A). The equilibration of radiolabel with AdoMet reached half-maximum within 24 min. Both the specific radioactivity of methionine and AdoMet approached an asymptote between 80 and 99% of the specific activity of extracellular methionine.

The fractional turnover of intracellular methionine (k_i) and AdoMet (k_2) was determined graphically from these equilibration curves using Equations A and B under "Materials and Methods," and these values are listed in Table 2. Included in Table 2 are the values from stimulated and unstimulated PBMC reported previously (7). The fractional turnover of L-methionine was similar in both stimulated and unstimulated Jurkat cells ranging from 2.5 to 4.0 min^-1, which is similar to that for PBMC (2.5 min^-1; Table 2). Upon cell activation, the AdoMet

MAT Subunit Composition in Resting and Activated Jurkat Cells. Recent data from our laboratory revealed the presence of a precursor of MAT in resting T-cells. This M, 68,000 precursor (λ) which has low MAT activity disappears when T-cells are stimulated with PHA while the α and α' subunits of the enzyme which have high MAT activity increase. The amount of the β subunit, on the other hand, remained constant throughout the course of stimulation. These changes in MAT subunits always occurred after IL-2 production and before DNA synthesis. It was of interest, therefore, to determine whether the induction of IL-2 production by Jurkat cells is also accompanied by a change in the relative amounts of MAT subunits. Cell extracts were prepared from resting and 24-h PHA-stimulated Jurkat cells and analyzed in Western blots for MAT subunits. In contrast to normal T-cells, Jurkat cells as well as ALL-2 cells lacked the λ form and only had the α, α', and β subunits (data not shown). Furthermore, when the cell extracts from the 24-h time point of the dose response experiment in Fig. 1 were analyzed by Western blotting, there was no change in the relative amounts of the different MAT α, α', and β subunits (Fig. 2). There was a slight but insignificant increase in the β subunit.
fractional turnover increased in PBMC by 38–200%; however, in Jurkat cells, AdoMet turnover was not significantly affected (Table 2). After activation of PBMC, the pool size of AdoMet also increased 4–10 fold compared to the unactivated cells (Table 2). On the other hand, the pool size of AdoMet in Jurkat cells remained constant at 140 pmol/10^6 cells, regardless of whether the cells were activated or not (Table 2). Although the fractional turnover of AdoMet in Jurkat cells was slightly less than that of PBMC, the fact that the AdoMet pool size in Jurkat cells (184 μM) was at least 23 times that of unstimulated PBMC (3–8 μM) indicated that AdoMet utilization in Jurkat cells was higher than in PBMC. In fact, AdoMet utilization in Jurkat cells was 5–7 μM/min compared to a value of 0.12–0.4 and 1.5 μM/min in unstimulated and stimulated PBMC, respectively (Table 2).

Correlation between Predicted and in Situ MAT Activity in Resting PBMC versus Stimulated PBMC and Jurkat Cells. Using the steady state kinetic model of Koth and Kredich (17) describing the MAT catalyzed reaction, we predicted the MAT activity in both PBMC and in Jurkat cells (Table 3). Enzyme activity can be predicted as a fraction of the V_max if the concentrations of the substrates and products of the reaction are known. The V_max itself was estimated empirically by assaying

Table 3 Predicted MAT reaction velocity and predicted MAT activity in PBMC and Jurkat T-cells

<table>
<thead>
<tr>
<th>Condition</th>
<th>PBMC (pmol/min/10^6 cells)</th>
<th>Jurkat cells (pmol/min/10^6 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+PHA</td>
<td>0.49</td>
<td>0.09</td>
</tr>
<tr>
<td>-PHA</td>
<td>0.28</td>
<td>0.09</td>
</tr>
<tr>
<td>In situ MAT activity*</td>
<td>0.36-0.49</td>
<td>0.56-0.76</td>
</tr>
</tbody>
</table>

*The predicted reaction velocity for PBMC was estimated using the steady state kinetic model of Koth and Kredich (17) shown below, where V_i is V_max, v is the initial velocity, A is the ATP concentration, B is the 1'-l-methionine concentration, P is the AdoMet concentration, Q is the pyrophosphate concentration, and R is the phosphate concentration.

\[
v = \frac{V_{i}AB(1 + Q + QR)}{K_{A}K_{S} + K_{A} + K_{B} + AB + C_{P} + C_{A}AP + C_{M}AP + C_{M}AP + C_{M}AP}
\]

The values obtained from the report of De La Rosa et al. (9) of 5 μM pyrophosphate, 5 mM phosphate, 1.5 mM ATP, 20 μM methionine, and 5 or 30 μM AdoMet were used in solving for v. The same values were used for predicting v in Jurkat cells, except that methionine was 100 μM (concentration in RPMI), and AdoMet was calculated to be 184 μM, based on a measured cell volume of 0.76 ml/10^6 cells.

Table 2 Comparison of AdoMet turnover, pool size, utilization, and MAT activity in PBMC

<table>
<thead>
<tr>
<th>Condition</th>
<th>PBMC</th>
<th>Jurkat cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>-PHA</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>+PHA</td>
<td>2.7-4.0</td>
<td>2.5-3.5</td>
</tr>
<tr>
<td>-PHA</td>
<td>0.029-0.06</td>
<td>0.025-0.03</td>
</tr>
<tr>
<td>+PHA</td>
<td>0.04-0.091</td>
<td>0.022-0.03</td>
</tr>
<tr>
<td>AdoMet pool size</td>
<td>1.4-3.4</td>
<td>17-23</td>
</tr>
<tr>
<td>μmol/min</td>
<td>3.3-8.1</td>
<td>25-33</td>
</tr>
<tr>
<td>AdoMet utilization</td>
<td>0.05-0.17</td>
<td>0.97-1.08</td>
</tr>
<tr>
<td>μmol/min</td>
<td>0.12-0.4</td>
<td>1.4-4.6</td>
</tr>
<tr>
<td>MAT activity (units/mg protein)</td>
<td>2-5^a</td>
<td>8-15</td>
</tr>
<tr>
<td>Jurkat cells</td>
<td>30-40</td>
<td>25-35</td>
</tr>
</tbody>
</table>

^a Turnover estimates for PBMC are from report of German et al. (7).

\[10^6\] cell volume were calculated based on cell volume for stimulated and unstimulated PBMC of 0.42 ml/10^6 cells and 0.69 ml/10^6 cells estimate by German et al. (7). The cell volume for the clone of Jurkat cells used in these studies was calculated to be 0.76 ml/10^6 cells.

^c MAT activities for PBMC are from De La Rosa et al. (9).
the enzyme under conditions of near-substrate saturation. Thus, the predicted MAT activity in situ is simply a product of the fraction of $V_{\text{max}}$ and the assayed MAT activity (which was corrected for 100% substrate saturation). Under steady state conditions it is expected that the rate of AdoMet utilization would equal the rate of input, i.e., the in situ MAT activity. In unstimulated PBMC, the in situ MAT activity was 7–3 fold lower than the predicted value. In contrast, in situ MAT activity in stimulated PBMC, as well as in Jurkat cells, was 2-fold higher than the predicted MAT activity (Table 3).

**DISCUSSION**

Abnormalities in methionine metabolism have been reported in a variety of cancer cells (18–21). Whether these changes are a consequence or cause of malignant transformation is not clear. However, inhibitors of AdoMet synthesis or inhibitors of DNA methylation have been shown to induce the differentiation of several transformed cell lines (22, 23). As a result of these reports, several laboratories have dedicated their efforts to studying the metabolism of AdoMet and designing specific inhibitors of its metabolism for use as chemotherapeutic agents (24–28). We are particularly interested in the role of AdoMet in the activation and differentiation of human T-lymphocytes and therefore have engaged in studies of the regulation of MAT activity in normal resting, activated, and transformed T-cells. Although several studies have compared MAT activity and AdoMet turnover in normal and malignant cell lines (21, 29, 30) the data are rather confusing. In some cases there was no difference in AdoMet metabolism, while in others, MAT specific activity was much higher in the transformed cells. We have found that both immortal T- and B-cell lines have much higher MAT specific activity than activated normal T- or B-lymphocytes or freshly isolated leukemic cells. However, MAT specific activity varied depending on the lineage of the leukemic cells.4

The Jurkat cell line has been used in many studies of T-cell activation because unlike other transformed T-cell lines, this line is able to respond to mitogenic stimuli by producing high levels of IL-2. These Jurkat cells, as shown in this study had a resting level of MAT activity of 30–40 units/mg protein and AdoMet utilization rate was 20–55 times that of unstimulated PBMC, and 3–5 times that of stimulated PBMC. Activation of Jurkat cells by PHA had no effect on either MAT activity or AdoMet turnover. This was not unique to this cell line because freshly isolated ALL-2 cells when stimulated to produce IL-2 behaved the same way. These results are similar to those obtained by Chiba et al. (31) with the promyelocytic cell line, HL-60 cells. These investigators showed that in these transformed cells MAT activity as well as the synthesis and pool size of AdoMet and $S$-adenosylhomocysteine were cell cycle independent. The data with Jurkat cells and HL-60 cells are quite different from that observed with resting PBMC, where activation triggers marked increase in both MAT activity, AdoMet pool size, and AdoMet turnover (7–9).

In PBMC, the change in AdoMet turnover is a relatively early event, occurring within 3–5 h of stimulation (7). This suggests that the increase in AdoMet level and turnover is not involved in early signal transduction but may be important in the initial stages of programming of cell activation. The increase in MAT activity in resting T-cells appear to follow the transformation of the less active $\lambda$ precursor form to the more active $\alpha/\alpha'$ form (10). Although this process is detected by 24 h of cell activation, it reaches a maximum at 48 h following IL-2 production and preceding DNA synthesis. It was not clear from our study whether the change in these subunits is a result or prerequisite of the cell cycle G1 to S transition. As shown in this study Jurkat and ALL-2 cells lack the $\lambda$ form, and the enzyme is constitutively present in the highly catalytic form $\alpha/\alpha'$. This may explain the lack of induction of enzyme activity despite induction of high levels of IL-2. Again the issue of whether this constitutively activated AdoMet metabolism is a cause or result of the process of malignant transformation requires further experimentation.

Clearly intact AdoMet metabolism is essential for normal T-cell proliferation. Previously, we demonstrated that inhibitors of AdoMet synthesis block T-cell blastogenesis in response to mitogenic and superantigenic stimulation (8). This is consistent with clinical and experimental reports that lymphocytic cells depend on AdoMet mediated reactions for immune function (32–35), which is not surprising since these cells undergo rapid differentiation. Indeed, the role of DNA methylation and the regulation of the expression of certain genes in lymphocytes has been shown in several studies (4–6). A comparison between the predicted and in situ MAT activity in resting and stimulated T-cells showed some interesting correlations. In unstimulated cells the in situ MAT activity was lower than the predicted activity, but in stimulated T-cells and Jurkat cells the situation was reversed. These observations can be interpreted in several ways: (a) Resting T-cells may have a less active form of MAT compared to stimulated cells; (b) an inhibitor of MAT may be present in resting but not in stimulated T-cells; (c) activation of T-cells may be accompanied by induction of a MAT activator(s). None of these possibilities are mutually exclusive, and further studies should reveal the mechanism of MAT regulation. However, the finding in this study that the differences in AdoMet metabolism are at least in part related to the presence of different forms of MAT in quiescent and actively dividing T-cells raises the possibility that this may be a feature that can be exploited for chemotherapy and immunomodulation.

Previous reports by Liau et al. (36) showed that tumor cells may have a different form of MAT. Such differences between normal and transformed cells provide the impetus for a more rigorous search for selective inhibitors of the various forms of MAT that can be of potential benefit in chemotherapy. Of particular interest in this regard is the report by Sufrin and Lombardini (37) which showed, based on studies with several substrates and inhibitors of MAT, that there are differences in the active-site of tumor versus normal isozymes of MAT. It may be possible, therefore, to synthesize inhibitors specific for the $\alpha/\alpha'$ but not the $\lambda$ form of MAT which can be used to target actively dividing T-cells without affecting normal T-cells.

**REFERENCES**


*J. De La Rosa, H. L. LeGros, and M. Kotb, manuscript in preparation.*
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