Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 19 (2008) 384–391
Journal of Nutritional Biochemistry

Ethanol inhibits methionine adenosyltransferase II activity and *S*-adenosylmethionine biosynthesis and enhances caspase-3-dependent cell death in T lymphocytes: relevance to alcohol-induced immunosuppression☆

Prachi T. Hote[a], Rashmita Sahoo[b], Tanvi S. Jani[a], Smita S. Ghare[c], Theresa Chen[a], Swati Joshi-Barve[b], Craig J. McClain[a,b,d], Shirish S. Barve[a,b,*]

[a]*Department of Pharmacology and Toxicology, University of Louisville Medical Center, Louisville, KY 40292, USA*
[b]*Department of Internal Medicine, University of Louisville Medical Center, Louisville, KY 40292, USA*
[c]*Department of Microbiology and Immunology, University of Louisville Medical Center, Louisville, KY 40292, USA*
[d]*Louisville VA Medical Center, Louisville, KY 40292, USA*

Received 26 February 2007; received in revised form 18 May 2007; accepted 23 May 2007

Abstract

An important aspect in alcohol abuse-associated immune suppression is the loss of T helper CD4$^+$ lymphocytes, leading to impairment of multiple immune functions. Our work has shown that ethanol can sensitize CD4$^+$ T lymphocytes to caspase-3-dependent activation-induced cell death (AICD). It has been demonstrated that the formation of *S*-adenosylmethionine (SAMe) catalyzed by methionine adenosyltransferase (MAT) II is essential for CD4$^+$ T-cell activation and proliferation. Since ethanol is known to affect SAMe metabolism in hepatocytes, we investigated the effect of ethanol on MAT II activity/expression, SAMe biosynthesis and cell survival in CD4$^+$ T lymphocytes. We demonstrate for the first time that ethanol at a physiologically relevant concentration (25 mM) substantially decreased the enzymatic activity of MAT II in T lymphocytes. Ethanol was observed to decrease the transcription of *MAT2A*, which encodes the catalytic subunit of MAT II and is vital for MAT II activity and SAMe biosynthesis. Furthermore, correspondent to its effect on MAT II, ethanol decreased intracellular SAMe levels and enhanced caspase-3-dependent AICD. Importantly, restoration of intracellular SAMe levels by exogenous SAMe supplementation considerably decreased both caspase-3 activity and apoptotic death in T lymphocytes. In conclusion, our data show that MAT II and SAMe are critical molecular components essential for CD4$^+$ T-cell survival that are affected by ethanol, leading to enhanced AICD. Furthermore, these studies provide a clinical paradigm for the development of much needed therapy using SAMe supplementation in the treatment of immune dysfunction induced by alcohol abuse.
© 2008 Elsevier Inc. All rights reserved.

Keywords: Ethanol; Methionine adenosyltransferase; *S*-Adenosylmethionine; CD4$^+$ T cells; Caspase-3; Activation-induced cell death

1. Introduction

Excessive alcohol consumption is known to have deleterious effects on the immune system [1–5]. Chronic alcohol administration in experimental animal systems leads to a decrease in the absolute number of CD4$^+$ T lymphocytes from the periphery and the spleen, as well as to a reduction in their immune function [6–12].

In human studies, alcohol-dependent patients have significantly reduced numbers of CD4$^+$ T lymphocytes, and the recovery of CD4$^+$ T-lymphocyte count after alcohol withdrawal has been noted in several studies, suggesting that ethanol can directly affect CD4$^+$ T-lymphocyte survival [13–18]. CD4$^+$ T lymphocytes are the central regulators of the immune system, controlling both cell-mediated and humoral immunity. While experimental and clinical studies

☆ This work was supported by National Institutes of Health grants AA014371 (S. Barve) and AA015970 (C.J. McClain) and by the Office of Dietary Supplements, National Institutes of Health.

* Corresponding author. Department of Medicine, Pharmacology and Toxicology, University of Louisville Medical Center, Louisville, KY 40202, USA. Tel.: +1 502 852 5245; fax: +1 502 562 4271.
E-mail address: shirish.barve@louisville.edu (S.S. Barve).

0955-2863/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.05.010

have documented that alcohol intake can cause depletion of $CD4^+$ T lymphocytes, the mechanisms underlying this alcohol effect are only beginning to be understood. Recently, our work has shown that the in vitro exposure of human $CD4^+$ T cells to physiologically relevant concentrations of ethanol enhances their susceptibility to Fas-induced and activation-induced cell death (AICD) by augmenting FasR- and TCR-CD3-mediated caspase-3 activation [19,20]. The present work was carried out to further clarify the mechanisms, and to identify the molecular components, involved in the ethanol-mediated enhancement of the AICD of $CD4^+$ T cells, which are the central regulators of the immune system, by controlling both cell-mediated and humoral immunity.

Intracellular *S*-adenosylmethionine (SAMe) is known to be critical for normal cell development and function. SAMe levels vary under different biologic conditions of differentiation and proliferation, and are regulated by biosynthesis and utilization [21]. SAMe is of pivotal importance to cellular metabolism and serves as the principal biologic donor of methyl groups in transmethylation reactions, thereby supporting the synthesis and modification of several key cellular components, including proteins, lipids, RNA and DNA. Moreover, SAMe controls essential metabolic pathways by regulating several important enzymatic reactions, including those involved in polyamine biosynthesis and single carbon metabolism [21]. Methionine adenosyltransferase (MAT) is a key enzyme in cellular metabolism because it catalyzes the only reaction that generates SAMe from L-methionine (L-Met) and ATP [21].

An important consequence of chronic alcohol abuse is the abnormal metabolism of hepatic SAMe due to decreased hepatic methionine adenosyltransferase (MAT–MAT I) expression and activity, resulting in hepatic SAMe deficiency and hepatotoxicity [22]. However, the effects of ethanol on nonhepatic MAT (MAT II) and SAMe levels in T lymphocytes have not been examined. In the case of T lymphocytes, both the SAMe pool size and the rate of SAMe utilization are known to increase upon T-cell activation [23]. A key mechanism for the increase in SAMe biosynthesis in T lymphocytes is increased transcription of *MAT2A*, which encodes the catalytic subunit of MAT II and is vital for MAT II activity and SAMe biosynthesis [24,25]. *MAT2A* is constitutively expressed in actively dividing/proliferating T cells, and its expression is inducible upon T-cell activation [26]. Hence, in the present work, we examined the effect of ethanol on MAT II activity expression and SAMe biosynthesis and its consequent impact on activation-induced $CD4^+$ T-cell death. Our data show for the first time that MAT II activity and SAMe biosynthesis are essential for T-cell survival and that their down-regulation by ethanol leads to enhancement of caspase-3-dependent apoptotic cell death. Importantly, exogenous SAMe supplementation significantly attenuates the ethanol-induced enhancement of caspase-3-dependent apoptotic cell death, indicating its potential therapeutic use in the treatment of alcohol-induced immune suppression.

2. Materials and methods

2.1. Cell culture and treatment

Jurkat T cells (clone E6-1; ATCC, Rockville, MD, USA) and MOLT-4 T cells (ATCC) were cultured in RPMI 1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 10 U/ml penicillin and 10 μg/ml streptomycin (Invitrogen Corporation, Carlsbad, CA, USA) at 37°C in a 5% CO_2 environment. Jurkat and MOLT-4 cells were resuspended in 1×10^6 cells/ml prior to treatment.

2.1.1. Peripheral blood lymphocyte isolation

After informed consent had been obtained from healthy nonalcoholic donors, fresh whole blood was drawn into Vacutainer tubes (Becton Dickinson Vacutainer; Becton Dickinson, Franklin Lakes, NJ) containing EDTA. Peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque-1077 (Sigma-Aldrich Chemicals, St. Louis, MO, USA). A total of 5×10^6 PBMCs/well were distributed into six-well plates (Corning, Inc., Costar, NY, USA) and allowed to adhere in a 5% CO_2 incubator at 37°C for 2 h in 2 ml of RPMI 1640. Nonadherent peripheral blood lymphocytes (PBLs) were removed from adherent cells (monocytes), washed carefully twice with prewarmed RPMI 1640 and resuspended at a concentration of 2×10^6 cells/ml.

2.2. Reagents and antibodies

Cell culture reagents were obtained from Invitrogen Corporation. Ethyl alcohol and phytohemagglutinin (PHA) were obtained from Sigma (St. Louis, MO, USA). SAMe, as 1,4-butanedisulphonate salt, was provided by Dr. R. O'Brian (Knoll Pharmaceuticals, Piscataway, NJ, USA) and Dr. G. Stramentionoli (Knoll Farmaceutici, Milan, Italy).

2.3. Reverse transcriptase–polymerase chain reaction and real-time PCR

Reverse transcriptase–polymerase chain reaction (RT-PCR) assays were used to assess *MAT2A* mRNA levels in Jurkat cells. Total RNA was isolated from treated cells after 3 h using TRIZOL (Invitrogen Corporation), and real-time PCR was performed as described elsewhere [27]. Specific primers were designed for human GAPDH and *MAT2A* using Primer3 software program. The following primers were used in real-time PCR:

GAPDH-RT-FP: 5′-TGGGCTACACTGAGCACCAG-3′
GAPDH-RT-RP: 5′-GGGTGTCGCTGTTGAAGTCA-3′
MAT2A-RT-FP: 5′-ACAATCTACCACCTACAGCCA-AGT-3′
MAT2A-RT-RP: 5′-GCATAAGAGACCTGAACAAGA-ACC-3′.

The parameter C_t (threshold cycle) was defined as the fraction cycle number at which fluorescence passed the threshold. The relative gene expression of *MAT2A* was

analyzed using the $2^{-\Delta\Delta Ct}$ method [28] by normalizing with GAPDH gene expression in all experiments.

2.4. MAT II activity and SAMe levels

MAT II activity was assayed in extracts prepared from cell pellets by three cycles of freeze thawing, as per the procedure described elsewhere [29], with minor modifications. MAT activity was assayed by using 20 μM L-Met, 5 mM ATP in 50 mM TES buffer (pH 7.4), 50 mM KCl, 15 mM $MgCl_2$, 0.3 mM EDTA and 4 mM DTT. One unit of MAT activity was defined as the amount of enzyme that catalyzes the formation of 1 nmol of SAMe in 1 h. SAMe catalyzed by MAT II, as well as intracellular SAMe levels, was assayed by reverse-phase high-performance liquid chromatography (HPLC) from deproteinized extracts prepared by using 4% metaphosphoric acid, as described elsewhere [30].

2.5. DNA fragmentation enzyme-linked immunosorbent assay analysis

Treated Jurkat cells, MOLT-4 cells or PBLs were lysed after 8 h to measure apoptosis. DNA fragmentation was quantitated using the Cell Death enzyme-linked immunosorbent assay (ELISA) kit (Roche, Indianapolis, IN, USA) according to a method described elsewhere [27].

2.6. Caspase-3 activity assay

Caspase-3 activity was quantified colorimetrically using cytoplasmic extracts prepared from Jurkat cells treated for 8 h. Caspase-3-like activity was analyzed using the Caspase-3 Fluorometric Assay Kit (R&D Systems, Inc., Minneapolis, MN, USA), as directed by the manufacturer.

2.7. Statistical analysis

Summary statistics are depicted as mean±S.E.M. Analysis of variance (ANOVA) was used to investigate the influence of ethanol levels on all outcome variables. For post-hoc pairwise comparisons, the Tukey–Kramer multiple comparison test was used (all figures except Fig. 3) to determine the significance (P value) of the effect of ethanol pretreatment on AICD. Differences were considered statistically significant at $P<.05$. Since intracellular levels of SAMe may not follow a Gaussian distribution, percent SAMe changes were measured (Fig. 3), and these data were examined using additional nonparametric tests (Friedman's one-way ANOVA), with the test significance level set at $P=.05$.

3. Results

The effect of ethanol exposure on MAT II, SAMe biosynthesis and AICD in $CD4^+$ T cells was primarily carried out at a physiologically relevant ethanol level of 25 mM. A blood ethanol concentration of 25 mM represents legally intoxicating levels and is easily achieved during human alcohol consumption. In all experiments involving ethanol treatment, ethanol concentrations were routinely measured posttreatment using the Sigma Diagnostics Alcohol Reagent (Sigma Diagnostics, St. Louis, MO, USA). Typically, under the experimental conditions used, a reduction in ethanol concentration by approximately 15–20% was consistently observed over the 24-h incubation period (data not shown).

3.1. Effect of ethanol on MAT II activity and intracellular SAMe levels

3.1.1. Ethanol decreases MAT II activity

Considering the importance of MAT II and SAMe biosynthesis in T-cell proliferation and function, the effect of ethanol exposure on MAT II activity in $CD4^+$ T cells was examined. Cells were untreated (control) or treated with varying concentrations of ethanol (2.5, 5, 10 and 25 mM) for 24 h. Cell extracts were examined for MAT II activity using a MAT enzymatic assay (as described in Materials and Methods) and expressed as picomoles of SAMe formed per microgram of (extract) protein. Ethanol was observed to decrease MAT II activity in T cells in a dose-dependent manner, with approximately 50% reduction occurring at 25 mM, which represents the physiologically relevant concentration of ethanol (Fig. 1).

3.1.2. Ethanol decreases MAT2A mRNA levels

Upon T-cell activation, a key mechanism for the increase in SAMe biosynthesis is increased transcription of *MAT2A*, which is the inducible gene partly responsible for increased MAT II activity. Since ethanol was observed to decrease MAT II activity, the effects of ethanol on *MAT2A* mRNA levels were evaluated before and after T-cell activation. Total RNA was isolated from cells exposed to ethanol (25 mM) with and without stimulation with PHA (5 μg/ml) for 3 h. *MAT2A* mRNA levels were assessed by real-time PCR.

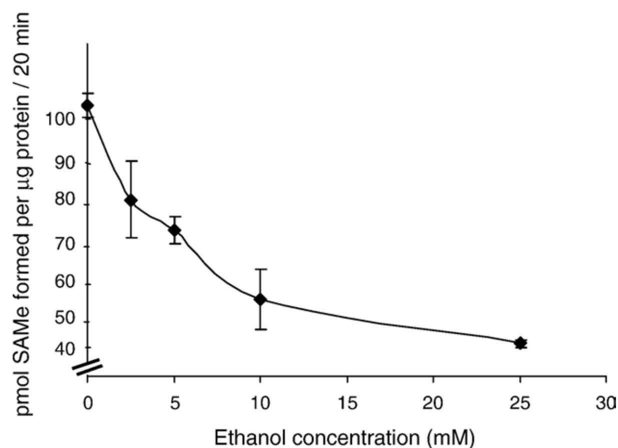

Fig. 1. Ethanol decreases MAT II activity. Jurkat cells were untreated (0) or treated with indicated concentrations of ethanol for 24 h. Cell extracts were prepared and MAT II activity was measured as described in Materials and Methods. Each point represents the mean±S.E.M. of three separate experiments.

Ethanol was found to significantly decrease both constitutive and PHA-inducible *MAT2A* mRNA expressions (Fig. 2).

3.1.3. Ethanol induces SAMe deficiency in $CD4^+$ T lymphocytes

Since MAT is a key enzyme that catalyzes the only reaction that generates SAMe, we then examined the effect of ethanol on intracellular levels of SAMe. Deproteinized extracts were prepared from cells exposed for 24 h to varying concentrations of ethanol (25, 50 and 100 mM) and SAMe levels determined by HPLC (Fig. 3). Since intracellular levels of SAMe may not follow a Gaussian distribution, percent SAMe changes were measured and analyzed using additional nonparametric tests (Friedman's one-way ANOVA). There was a significant difference in percent SAMe levels between no ethanol (0 mM) and increasing concentrations of ethanol (25–100 mM) ($P<.05$). All results were consistent; importantly, a mean decrease of 30% in intracellular SAMe levels was observed in T cells exposed to a physiologically relevant concentration (25 mM) of ethanol after a period of 24 h. These results showed that correspondent to its effect on MAT II activity and expression, ethanol exposure led to a dose-dependent decrease in intracellular SAMe concentrations, causing SAMe deficiency in T cells (Fig. 3).

3.2. Effect of the pharmacologic inhibition of MAT II on intracellular SAMe levels and T-lymphocyte survival

3.2.1. MAT II inhibition and SAMe deficiency enhance T-lymphocyte AICD

Our previous work has shown that physiologically relevant concentrations of ethanol (25 mM) enhance caspase-3-dependent AICD of $CD4^+$ T lymphocytes. Therefore, to establish the causative role of MAT II inhibition and resultant SAMe deficiency in this ethanol-mediated enhancement of T-cell apoptotic death, MAT II activity was inhibited using cycloleucine (1-aminocyclopentane-1-carboxylic acid), a cyclic analogue of methionine that acts as a specific inhibitor of MAT [31–33]. Cells were untreated (control) or treated with varying concentrations of cycloleucine (10–60 mM) for 4 h. MAT II activity was measured in cell extracts. Further intracellular SAMe levels were measured in control cells (untreated) or in cells treated with cycloleucine (10, 20 and 40 mM) for 4 h. SAMe levels were measured by HPLC. As expected, cycloleucine treatment inhibited MAT II activity in a dose-dependent manner (Fig. 4A) and consequently decreased cellular SAMe levels (Fig. 4B). Such inhibition of MAT II activity and lowering of cellular SAMe levels were observed to significantly induce and enhance the AICD of T lymphocytes as documented by quantitative DNA fragmentation analysis (Fig. 5). These data show that similar to alcohol, pharmacologic induction of SAMe deficiency sensitizes $CD4^+$ T cells to undergo enhanced AICD. Taken together, these data strongly implicate that MAT II activity and SAMe are critical components of T-lymphocyte survival that are affected by ethanol.

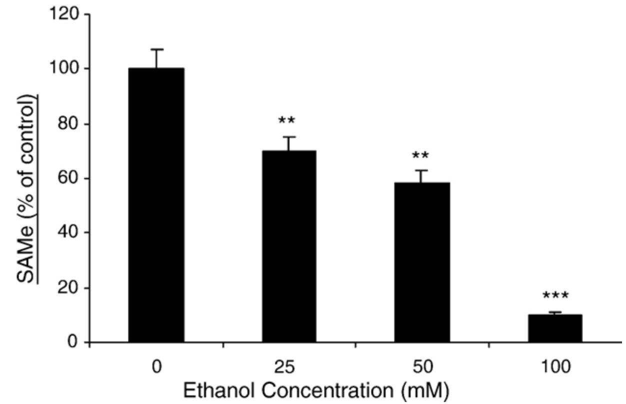

Fig. 3. Ethanol decreases intracellular SAMe levels in a dose-dependent manner. Jurkat cells were untreated (0) or treated with increasing concentrations of ethanol (25, 50 and 100 mM) for 24 h. Cell extracts were prepared and SAMe levels were measured by a process described in Materials and Methods. Results are presented as the mean±S.E.M. of three separate experiments. $^{**}P<.01$. $^{***}P<.001$, versus untreated (0).

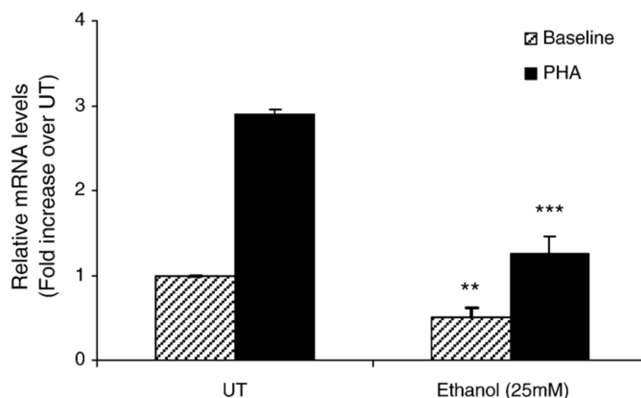

Fig. 2. Ethanol decreases *MAT2A* gene expression. Quantification of *MAT2A* mRNA levels by real-time PCR. Jurkat cells were either untreated (UT) or treated with 25 mM ethanol, with or without stimulation by PHA (5 μg/ml; solid bars), for 3 h. Results are expressed as the mean±S.E.M. of three separate experiments. $^{**}P<.05$, ethanol alone versus untreated (UT). $^{***}P<.001$, ethanol+PHA versus PHA alone.

3.3. Effect of SAMe supplementation on the ethanol-mediated enhancement of activation-induced T-lymphocyte apoptotic death

3.3.1. Exogenous SAMe supplementation increases cellular SAMe levels

Data obtained from ethanol- and cycloleucine-treated cells showed that a decline in MAT II activity and consequent SAMe deficiency can significantly enhance the susceptibility of T cells to apoptotic death. Accordingly, the effects of SAMe supplementation on ethanol-treated $CD4^+$ T lymphocytes were evaluated. Initially, the effect of exogenous SAMe supplementation on intracellular SAMe levels was examined. Cells were treated with increasing concentrations of SAMe (0.25, 0.5 and 1.0 mM) for 4 h, and

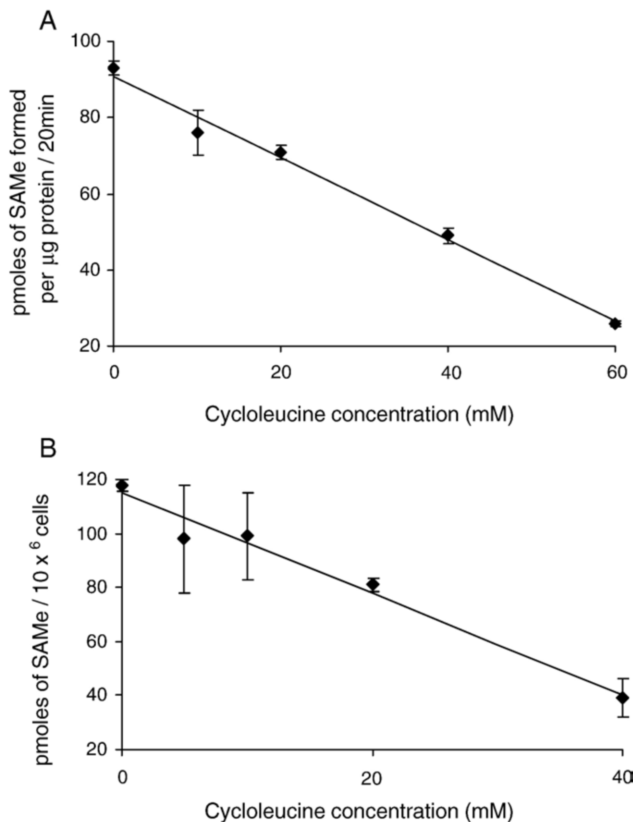

Fig. 4. Pharmacologic inhibition of MAT II and SAMe biosynthesis enhances T-lymphocyte AICD. (A) Cycloleucine decreases MAT II activity in a dose-dependent manner. Jurkat cells were untreated (0) or treated with indicated concentrations of cycloleucine for 4 h. Cell extracts were prepared and MAT II activity was measured as described in Materials and Methods. Each point represents the mean±S.E.M. of three separate experiments. (B) Cycloleucine induces SAMe deficiency in a dose-dependent manner. Cell (Jurkat) extracts were prepared from cells that were either untreated (0) or treated with indicated concentrations of cycloleucine for 4 h, and intracellular SAMe levels were measured. Results are shown as the mean±S.E.M. of three separate experiments.

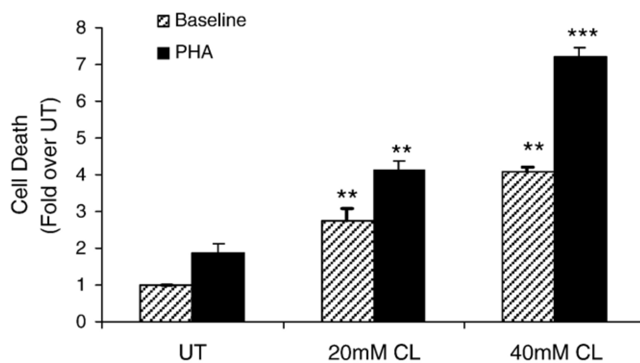

Fig. 5. Inhibition of MAT II by cycloleucine leads to activation-induced apoptotic death of CD^+ 4 T lymphocytes. DNA fragmentation analysis of Jurkat cells untreated (UT) or pretreated with cycloleucine (20 and 40 mM CL) for 4 h with or without stimulation by PHA (5 µg/ml; solid bars) for 6 h. Data are normalized to untreated and expressed as the mean±S.E.M. of three separate experiments. ***P*<.01, cycloleucine (CL) alone versus untreated (UT). ****P*<.001, CL+PHA versus PHA alone.

intracellular SAMe pools were measured in deproteinized extracts by HPLC. Exogenous SAMe supplementation significantly increased intracellular SAMe levels in a dose-dependent manner, with maximal concentrations achieved at 1.0 mM (Fig. 6). Hence, subsequent studies evaluating the effect of SAMe supplementation on ethanol-treated cells were carried out at 1.0 mM.

3.3.2. SAMe supplementation decreases ethanol-mediated increase in caspase-3 activity and attenuates AICD in T lymphocytes

Our earlier data showed that ethanol-induced enhancement in the susceptibility of $CD4^+$ T lymphocytes to AICD is mediated by an increase in caspase-3 activation. Hence, to further elucidate the role of SAMe deficiency as an underlying cause in ethanol-induced $CD4^+$ T-cell toxicity, the effect of SAMe supplementation was assessed.

Cells were pretreated with SAMe (1.0 mM) for 4 h, followed by ethanol treatment (25 mM) for 24 h with or without PHA stimulation for 8 h. As seen before, ethanol augmented PHA-inducible caspase-3 activity, which was significantly attenuated by SAMe supplementation (Fig. 7A). Additionally, SAMe supplementation significantly attenuated ethanol-mediated enhancement in AICD as documented by DNA fragmentation analysis (Fig. 7B). To ensure that the induction of apoptosis by MAT II inhibition was not cell line-specific (i.e., restricted to Jurkat T cells), we tested the effect of MAT II inhibition on another human leukemic T-cell line, namely, MOLT-4. A similar attenuation of ethanol-mediated apoptotic death by SAMe was observed in MOLT-4 T cells (Fig. 7C).

3.3.3. SAMe supplementation attenuates the ethanol-induced enhancement of AICD in human PBLs

Since ethanol exposure enhances AICD in primary human lymphocytes, the effect of SAMe supplementation on human PBLs isolated from six healthy (nonalcoholic) volunteers was also evaluated. PBLs were pretreated with SAMe (1.0 mM) for 4 h, followed by ethanol treatment

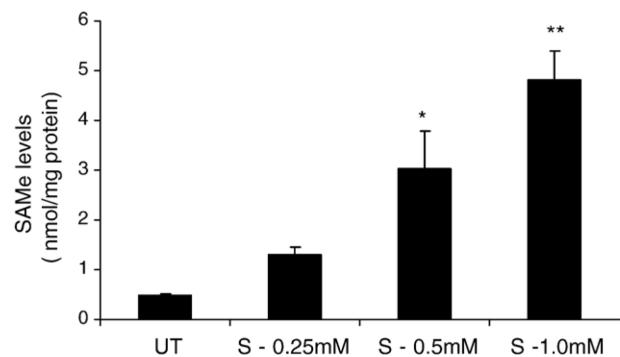

Fig. 6. Exogenous SAMe supplementation increases cellular SAMe levels. Jurkat cells were untreated (UT) or treated with increasing concentrations of SAMe (0.25, 0.5 and 1 mM) for 4 h. Cell extracts were prepared and SAMe levels were measured by HPLC. Results are presented as the mean±S.E.M. of three separate experiments. **P*<.05. ***P*<.01, versus untreated (UT).

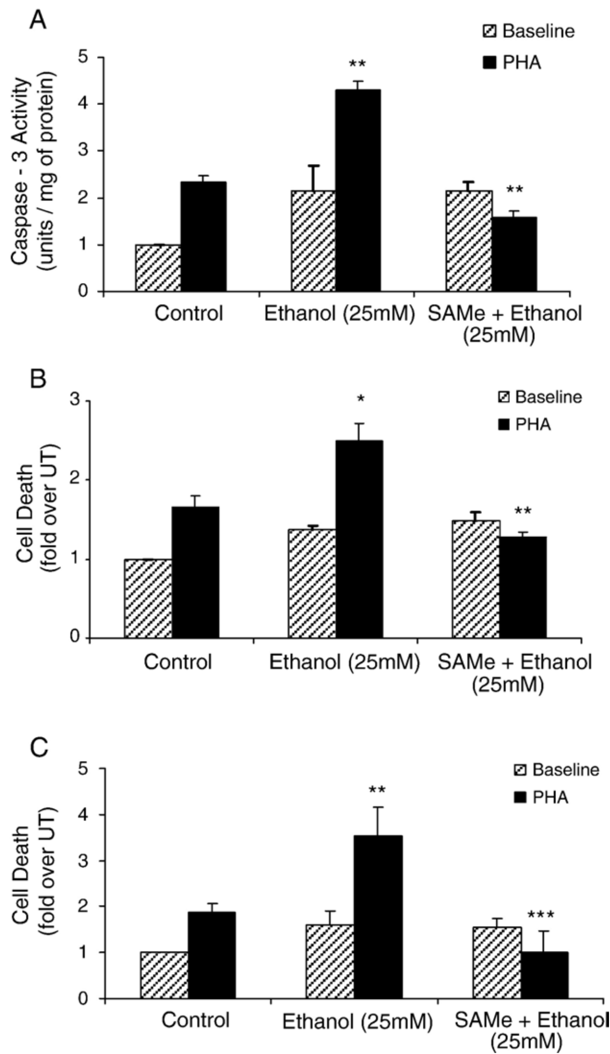

Fig. 7. SAMe supplementation decreases the ethanol-mediated increase in caspase-3 and attenuates AICD in T lymphocytes. (A) Quantification of ethanol-mediated caspase-3 activity. Jurkat cells were untreated (control) or pretreated with SAMe (1 mM) for 4 h and then exposed to 25 mM ethanol for 24 h with or without stimulation by PHA (5 μg/ml; solid bars) for 8 h. Cytoplasmic extracts were isolated and assayed for caspase-3 activity. Results are presented as the mean±S.E.M. of three separate experiments and expressed as fold over untreated (set to 1). $^{**}P<.01$, ethanol+PHA versus PHA alone; SAMe+ethanol+PHA versus ethanol+PHA. (B) DNA fragmentation analysis of T (Jurkat) cells that were treated in a similar manner as mentioned above (A). Results are presented as the mean±S.E.M. of three separate experiments and expressed as fold over untreated (set to 1). $^{*}P<.05$, ethanol+PHA versus PHA alone. $^{**}P<.01$, SAMe+ethanol+PHA versus ethanol+PHA. (C) Exogenous SAMe supplementation significantly attenuates ethanol-mediated AICD in MOLT-4 T cells. Cells (MOLT-4) were untreated (control) or pretreated with SAMe (1 mM) for 4 h and then exposed to 25 mM ethanol for 24 h with or without stimulation by PHA (5 μg/ml; solid bars) for 8 h. AICD was quantified from cell extracts using Cell Death Detection ELISA. Results are presented as the mean±S.E.M. of three separate experiments and expressed as fold over untreated (set to 1). $^{**}P<.01$, ethanol+PHA versus PHA alone. $^{***}P<.001$, SAMe+ethanol+PHA versus ethanol+PHA.

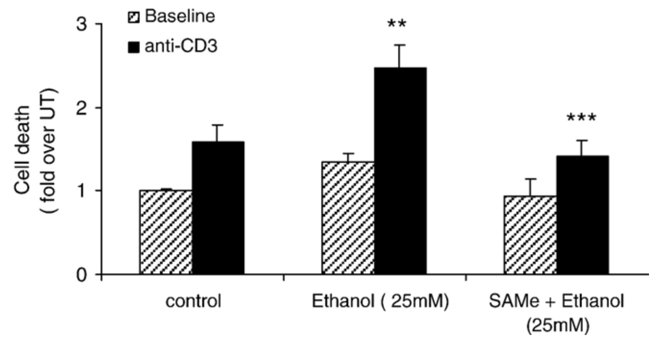

Fig. 8. SAMe supplementation attenuates the ethanol-induced enhancement of AICD in human PBLs. PBLs were untreated (control) or pretreated with SAMe (1 mM) for 4 h and then exposed to 25 mM ethanol for 24 h with or without stimulation with plate-bound anti-CD3 antibody (solid bars) for 8 h. AICD was measured by quantifying DNA fragmentation with Cell Death Detection ELISA. Results are presented as the mean±S.E.M. of six healthy (nonalcoholic) volunteers and expressed as fold over untreated (set to 1). $^{**}P<.01$, ethanol+PHA versus PHA alone. $^{***}P<.001$, SAMe+ethanol+PHA versus ethanol+PHA.

(25 mM) for 24 h with or without stimulation with plate-bound anti-CD3 antibody (5 μg/ml; solid bars) for 8 h. AICD in human PBLs was measured by quantifying DNA fragmentation with Cell Death Detection ELISA. In agreement with our earlier findings, ethanol exposure enhanced AICD in human PBLs. Importantly, SAMe pretreatment, similar to both Jurkat and MOLT-4 $CD4^+$ T cells, significantly attenuated AICD in primary human PBLs (Fig. 8). Additionally, matching results were obtained in ethanol- and SAMe-treated human PBLs that were activated with PHA (5 μg/ml; data not shown). These findings strongly indicate that inhibition of MAT II activity/expression and the resultant SAMe deficiency are critical molecular events mediated by ethanol exposure that lead to the augmentation of activation-induced apoptotic death in $CD4^+$ T lymphocytes.

4. Discussion

A critical factor in alcohol-induced immunosuppression is the loss of $CD4^+$ T lymphocytes, which could lead to impairment of multiple immune functions. While experimental and clinical studies have documented that chronic alcohol intake can cause a reduction in the number of $CD4^+$ T lymphocytes, the underlying mechanisms are only beginning to be understood. Our earlier studies showed that physiologically relevant concentrations of ethanol could induce and enhance caspase-3-dependent apoptotic death in human $CD4^+$ T lymphocytes by sensitizing them to AICD [19]. The present work was aimed at identifying molecular events that could be involved in this ethanol-mediated sensitization of $CD4^+$ T lymphocytes to AICD.

The results from this study document for the first time that the exposure of $CD4^+$ T lymphocytes to ethanol can

significantly decrease MAT II activity in a dose-dependent manner, with a concomitant decrease in intracellular SAMe levels. Further analysis showed that ethanol at physiologically relevant concentrations (25 mM) substantially decreases the transcription of *MAT2A*, which encodes the catalytic subunit of MAT II and is the vital component of MAT II activity and SAMe biosynthesis. These results are clinically significant in the context of immunotoxicity observed in alcohol-dependent patients, who also have hepatic SAMe deficiency and hepatotoxicity due to decreased hepatic methionine adenosyl transferase (MAT I/III) activity and expression.

T-cell activation leads to an increment in both SAMe pool size and SAMe utilization, and inhibition of SAMe synthesis blocks T-cell proliferation [34]. Additionally, ethanol enhances caspase-3-dependent apoptotic death in T lymphocytes. Taken together, our present data strongly suggest that ethanol-mediated decrease in MAT II expression and activity and consequent SAMe deficiency play a significant role in the development of the ethanol-mediated susceptibility of $CD4^+$ T cells to AICD. This is further supported by observations that inhibition of MAT II activity and decrease in intracellular SAMe levels by a selective pharmacologic inhibitor (cycloleucine) also induce and enhance activation-induced apoptotic death in T lymphocytes. The causal role of MAT II inhibition and the ensuing SAMe deficiency in ethanol-mediated $CD4^+$ T-cell death are further supported by data obtained from SAMe supplementation studies. The restoration of intracellular SAMe levels achieved by exogenous SAMe supplementation, which bypasses the requirement of MAT II activity, was observed to considerably counter the cytotoxic effects of ethanol by decreasing both caspase-3 activity and AICD in T cells. Importantly, the protective effects of SAMe supplementation observed in two different $CD4^+$ T-cell lines (Jurkat and MOLT-4) were also recapitulated in human PBLs.

Overall, these data strongly implicate a survival role for MAT II and SAMe in activated T cells. Moreover, the present work identifies *MAT2A* gene to be directly associated with T-cell survival and supports the notion that MAT-II-dependent SAMe biosynthesis and the consequent transmethylation reactions controlled by SAMe, either directly or indirectly, prevent the activation-induced apoptotic signaling that leads to caspase-3 activation and T-cell death. The mechanisms underlying SAMe-mediated regulation of apoptotic signaling and activation-induced T-cell death are currently under investigation.

In conclusion, we have identified MAT II and SAMe as critical molecular components required for $CD4^+$ T-cell survival that are diminished by ethanol, leading to an increase in activation-induced apoptotic death. Based on these findings, we postulate that investigation of the effects of alcohol on $CD4^+$ T-cell MAT II activity, SAMe levels and survival will provide important information on mechanisms of alcohol-induced immunosuppression. Additionally, attenuation of ethanol-induced $CD4^+$ T-cell apoptotic death by exogenous SAMe supplementation provides “proof of concept” for the potential therapeutic use of SAMe in treating immune dysfunction associated with chronic alcohol abuse in alcohol-dependent patients with and without other immunosuppressive disorders such as HIV/hepatitis C virus and diabetes.

References

[1] Adams HG, Jordan C. Infections in the alcoholic. Med Clin North Am 1984;68:179–200.

[2] MacGregor RR. Alcohol and immune defense. JAMA 1986;19:1474–9.

[3] Smith FE, Palmer DL. Alcoholism, infection and altered host defenses: a review of clinical and experimental observations. J Chronic Dis 1976;29:35–49.

[4] Spies CD, von Dossow V, Eggers V, Jetschmann G, El-Hilali R, Egert J, et al. Altered cell-mediated immunity and increased postoperative infection rate in long-term alcoholic patients. Anesthesiology 2004;100:1088–100.

[5] Waldschmidt TJ, Cook RT, Kovacs EJ. Alcohol and inflammation and immune responses: summary of the 2005 Alcohol and Immunology Research Interest Group (AIRIG) meeting. Alcohol 2006;38:121–5.

[6] Helm RM, Wheeler G, Burks AW, Hakkak R, Badger TM. Flow cytometric analysis of lymphocytes from rats following chronic ethanol treatment. Alcohol 1996;13:467–71.

[7] Chadha KC, Stadler I, Albini B, Nakeeb SM, Thacore HR. Effect of alcohol on spleen cells and their functions in C57BL/6 mice. Alcohol 1991;8:481–5.

[8] Shellito JE, Olariu R. Alcohol decreases T-lymphocyte migration into lung tissue in response to *Pneumocystis carinii* and depletes T-lymphocyte numbers in the spleens of mice. Alcohol Clin Exp Res 1998;22:658–63.

[9] Peterson JD, Herzenberg LA, Vasquez K, Waltenbaugh C. Glutathione levels in antigen-presenting cells modulate Th1 versus Th2 response patterns. Proc Natl Acad Sci U S A 1998;17:3071–6.

[10] Saad AJ, Jerrells TR. Flow cytometric and immunohistochemical evaluation of ethanol-induced changes in splenic and thymic lymphoid cell populations. Alcohol Clin Exp Res 1991;15:796–803.

[11] Jerrells TR, Saad AJ, Domiati-Saad R. Effects of ethanol on parameters of cellular immunity and host defense mechanisms to infectious agents. Alcohol 1992;9:459–63.

[12] Padgett EL, Sibley DA, Jerrells TR. Effect of adrenalectomy on ethanol-associated changes in lymphocyte cell numbers and subpopulations in thymus, spleen, and gut-associated lymphoid tissues. Int J Immunopharmacol 2000;22:285–98.

[13] Young GP, Van der Weyden MB, Rose IS, Dudley FJ. Lymphopenia and lymphocyte transformation in alcoholics. Experientia 1979;35:268–9.

[14] Roselle GA, Mendenhall CL, Grossman CJ, Weesner RE. Lymphocyte subset alterations in patients with alcoholic hepatitis. J Clin Lab Immunol 1988;26:169–73.

[15] Pol S, Artru P, Thepot V, Berthelot P, Nalpas B. Improvement of the $CD4^+$ cell count after alcohol withdrawal in HIV-positive alcoholic patients. AIDS 1996;10:1293–4.

[16] Laso FJ, Madruga JI, Lopez A, Ciudad J, Alvarez-Mon M, San Miguel J, et al. Distribution of peripheral blood lymphoid subsets in alcoholic liver cirrhosis: influence of ethanol intake. Alcohol Clin Exp Res 1996;20:1564–8.

[17] Gheorghiu M, Bara C, Pasarica D, Brasoveanu L, Bleotu C, Toparceanu F, et al. Ethanol-induced dysfunction of hepatocytes and leukocytes in patients without liver failure. Roum Arch Microbiol Immunol 2004;63:5-33.

[18] Kazbariene B, Krikstaponiene A, Monceviciute-Eringiene E. Disturbance of human immunohomeostasis by environmental pollution

and alcohol consumption. Acta Microbiol Immunol Hung 2006;53: 209–18.

[19] Kelkar S, Dong Q, Xiao Y, Joshi-Barve S, McClain CJ, Barve SS. Ethanol enhances activation-induced caspase-3 dependent cell death in T lymphocytes. Alcohol Clin Exp Res 2002;26:363–70.

[20] Donohue Jr TM, Clemens DL, Galli A, Crabb D, Nieto N, Kato J, et al. Use of cultured cells in assessing ethanol toxicity and ethanol-related metabolism. Alcohol Clin Exp Res 2001;25:87S–93S.

[21] Mato JM, Corrales FJ, Lu SC, Avila MA. *S*-Adenosylmethionine: a control switch that regulates liver function. FASEB J 2002;16:15–26.

[22] Cabrero C, Duce AM, Ortiz P, Alemany S, Mato JM. Specific loss of the high-molecular-weight form of *S*-adenosyl-L-methionine synthetase in human liver cirrhosis. Hepatology 1988;8:1530–4.

[23] German DC, Bloch CA, Kredich NM. Measurements of *S*-adenosylmethionine and L-homocysteine metabolism in cultured human lymphoid cells. J Biol Chem 1983;258:10997–1003.

[24] LeGros Jr HL, Geller AM, Kotb M. Differential regulation of methionine adenosyltransferase in superantigen and mitogen stimulated human T lymphocytes. J Biol Chem 1997;272:16040–7.

[25] Zeng Zeng Z, Yang H, Huang ZZ, Chen C, Wang J, Lu SC. The role of c-Myb in the up-regulation of methionine adenosyltransferase 2A expression in activated Jurkat cells. J Biochem 2001;353:163–8.

[26] Kotb M, Kredich NM. *S*-Adenosylmethionine synthetase from human lymphocytes. Purification and characterization. J Biol Chem 1985;260: 3923–30.

[27] Uriarte SM, Joshi-Barve S, Song Z, Sahoo R, Gobejishvili L, Jala VR, et al. Akt inhibition upregulates FasL, downregulates c-FLIPs and induces caspase-8-dependent cell death in Jurkat T lymphocytes. Cell Death Differ 2005;12:233–42.

[28] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. Methods 2001;25:402–8.

[29] De La Rosa J, LeGros Jr HL, Geller AM, Kotb M. Changes in the relative amount of subunits of methionine adenosyltransferase in human lymphocytes upon stimulation with a polyclonal T cell mitogen. J Biol Chem 1992;267:10699–704.

[30] Song Z, Chen T, Deaciuc IV, Uriarte S, Hill D, Barve S, et al. Modulation of endotoxin stimulated interleukin-6 production in monocytes and Kupffer cells by *S*-adenosylmethionine (SAMe). Cytokine 2004;28:214–23.

[31] Lombardini JB, Coulter AW, Talalay P. Analogues of methionine as substrates and inhibitors of the methionine adenosyltransferase reaction. Deductions concerning the conformation of methionine. Mol Pharmacol 1970;6:481–99.

[32] Sufrin JR, Coulter AW, Talalay P. Structural and conformational analogues of L-methionine as inhibitors of the enzymatic synthesis of *S*-adenosyl-L-methionine: IV. Further mono-, bi- and tricyclic amino acids. Mol Pharmacol 1979;15:661–77.

[33] Lombardini JB, Sufrin JR. Chemotherapeutic potential of methionine analogue inhibitors of tumor-derived methionine adenosyltransferases. Biochem Pharmacol 1983;32:489–95.

[34] Kotb M, Dale JB, Beachey EH. Stimulation of *S*-adenosylmethionine synthetase in human lymphocytes by streptococcal M protein. J Immunol 1987;139:202–6.