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Immunology

Inhibition of lymphocyte-mediated cytolysis by 3-deazaadenosine: Evidence for a methylation reaction essential to cytolysis

(*S*-adenosylhomocysteine/*S*-3-deazaadenosylhomocysteine/*S*-adenosylmethionine/adenosylhomocysteinase/L-homocysteine thiolactone)

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ABSTRACT **3-Deazaadenosine (deazaAdo) inhibits lymphocyte-mediated cytolysis *in vitro* at micromolar concentrations and is potentiated markedly in this activity by L-homocysteine thiolactone. DeazaAdo alone causes a rapid, dose-dependent buildup of *S*-[^{3}H]adenosylhomocysteine (AdoHcy) and *S*-[^{3}H]adenosylmethionine in cytolytic lymphocytes labeled with L-[2-^{3}H]methionine; smaller amounts of *S*-3-[^{3}H]deazaadenosylhomocysteine (deazaAdoHcy) are also formed in these cells. The simultaneous addition of deazaAdo and L-homocysteine thiolactone to the lymphocytes results in a massive intracellular accumulation of deazaAdoHcy. Both the inhibition of lymphocyte-mediated cytolysis and the cellular accumulation of [^{3}H]AdoHcy caused by deazaAdo alone are reversed rapidly by removal of drug from the medium. However, the inhibition of cytolysis and the large cellular buildup of deazaAdoHcy resulting from treatment of the lymphocytes with deazaAdo plus L-homocysteine thiolactone are dissipated more slowly under these same conditions. Unlike adenosine, deazaAdo is not potentiated in its inhibition of lymphocyte-mediated cytolysis by Ro 20-1724 [4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone], an inhibitor of cyclic AMP phosphodiesterase, and has little or no effect upon the level of lymphocytic cyclic AMP. DeazaAdo is not metabolized detectably to 5′-nucleotides in the lymphocytes and does not cause a decrease in the pool sizes of CTP, UTP, ATP, or GTP. Both AdoHcy and deazaAdoHcy have been reported to be powerful inhibitors of a variety of *S*-adenosylmethionine-utilizing methyltransferases. The present results, therefore, indicate that the effect of deazaAdo upon lymphocyte-mediated cytolysis is due ultimately to the inhibition of an unidentified but crucial methyltransferase within the cytolytic lymphocytes and provide an insight into the biochemical processes involved in lymphocyte-mediated cytolysis.**

Among their many important functions in cellular immunity, sensitized thymus-derived lymphocytes can effect the specific lysis of antigen-bearing target cells; this phenomenon, lymphocyte-mediated cytolysis (LMC), may play a substantial role in tumor and graft rejection (for reviews see refs. 1–3). LMC can be studied conveniently and rapidly (1–4 hr) *in vitro* by monitoring the release of appropriate radioisotopes from radiolabeled target cells (4). By such *in vitro* experimental systems, LMC has been found to be a multistage process wherein each specifically sensitized lymphocyte binds transiently to a target cell, "programs" this latter cell for imminent lysis, and then departs from the doomed cell in search of other similar target cells (5, 6). Cytolysis proceeds linearly with time and is first order with respect both to cytolytic lymphocytes and to target cells (7). In the LMC assay, radioactive markers are released from target cells sequentially according to molecular weight, smaller markers being released first (8). Treatment of target cells with cytolytic lymphocytes initially causes swelling prior to the explosive lysis of the target cells (9). LMC is dependent upon temperature (1), pH (10), divalent cations (1), and metabolic energy (11, 12) and is inhibited by agents that interact with microfilaments and microtubules (13). Structurally diverse agents, such as prostaglandin E_1 (14), isoproterenol (15), cholera toxin (16), and adenosine (17), which cause an elevation of lymphocytic cyclic AMP (cAMP), also inhibit LMC. By contrast, cholinergic agonists have been reported to stimulate LMC (18). The cytolytic activity of lymphocytes appears to be insensitive to inhibition of RNA (19) or protein synthesis (20).

Although much has been learned concerning LMC, the precise mechanism of this immunological process remains a mystery. Among the different hypotheses advanced to explain LMC, Henney (2) has suggested that the cytolytic lymphocytes secrete a toxic factor into or onto the target cell during the contact stage of LMC. Henkart and Blumenthal (21) have provided indirect evidence for insertion of channel-forming molecules into the membrane of the target cell, thereby allowing salts, water, and other small molecules to flow across the cell membrane. Seeman (9) has proposed that the cytolytic lymphocytes, during detachment from the target cells, produce a tangential shear force on the target-cell membrane sufficient to allow salt leakage and subsequent colloid osmotic lysis. The present report describes evidence indicating that an unidentified, *S*-adenosylmethionine (AdoMet)-utilizing methyltransferase within the cytolytic lymphocytes may play an important role in the mechanism of immune cytolysis.

MATERIALS AND METHODS

Materials. 3-Deazaadenosine (deazaAdo) was provided by O. H. Viveros of these Laboratories. L-[2-^{3}H]Methionine (2.9 Ci/mmol) was purchased from Amersham/Searle. *S*-3-Deazaadenosylhomocysteine (deazaAdoHcy) was a gift from R. T. Borchardt (University of Kansas) and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) was provided by H. Sheppard (Hoffmann–La Roche). *erythro*-9-(2-Hydroxy-3-nonyl)adenine hydrochloride was synthesized at the Wellcome Research Laboratories. AdoMet (iodide salt), *S*-adenosylhomocysteine (AdoHcy), L-homocysteine thiolactone (Hcy), adenosine, and 2-chloroadenosine were from Sigma. Mice, C57BL leukemia EL4, $Na_2{}^{51}CrO_4$, Dulbecco's phosphate-buffered saline, and fetal calf serum were obtained from sources identified previously (22).

Cells. C57BL leukemia EL4 was maintained, harvested, and labeled with $Na_2{}^{51}CrO_4$ (22). Cytolytic lymphocytes were obtained from CD-1 mice as reported earlier (23). Dulbecco's

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Abbreviations: AdoHcy, *S*-adenosylhomocysteine; AdoMet, *S*-adenosylmethionine; deazaAdo, 3-deazaadenosine; deazaAdoHcy, *S*-3-deazaadenosylhomocysteine; Hcy, L-homocysteine thiolactone; LMC, lymphocyte-mediated cytolysis; IC_{50}, concentration of drug required to produce 50% inhibition of a particular process; Ro 20-1724, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone; cAMP, cyclic AMP.

phosphate-buffered saline supplemented with 10% fetal calf serum (heat-inactivated) was used as the medium for all cell incubations.

***In Vitro* Assay of LMC.** The *in vitro* assay of cytolysis has been described (17, 22). Briefly, this assay determined the amount of ^{51}Cr released during a 70-min incubation at 37°C of ^{51}Cr-labeled EL4 cells and cytolytic lymphocytes. With a 1:1 ratio of cytolytic lymphocytes to target cells, approximately 25% cellular lysis takes place in the control (uninhibited) assays. Unless specified otherwise, drugs were added to the cytolytic lymphocytes only at the start of the LMC assay.

Studies of Drug Effects on Lymphocyte Metabolism of [2-^{3}H]Methionine. Cytolytic lymphocytes (2.5–3.3 × 10^8 cells in 10 ml of medium) were preincubated for 60 min at 37°C with 100 μCi of L-[2-^{3}H]methionine and were then chilled and washed. These cells were resuspended in fresh medium to a density of 3.1–6.1 × 10^6 cells per ml, and 5.0-ml portions of these cells were supplemented with the specified agents and incubated for the indicated times at 37°C. All experiments were performed in duplicate. Cells were extracted with 5.0 ml of cold 1.0 M perchloric acid containing 2.0 μM 2-chloroadenosine as a recovery marker. The neutralized extracts were evaporated to dryness, reconstituted in 300 μl of 50 mM ammonium phosphate (pH 6.0), and stored at −20°C until their analysis by reversed-phase high-pressure liquid chromatography.

Other Studies with Cytolytic Lymphocytes. In metabolic studies of deazaAdoHcy formation from deazaAdo and Hcy, cytolytic lymphocytes (1.8 × 10^7 cells per 5.0 ml of medium) were supplemented with saline or drug(s) and incubated for the specified times at 37°C. Incubations were terminated, cells were extracted, and extracts were processed exactly as described above. The effect of drugs on levels of cAMP and ribonucleoside 5′-phosphates in cytolytic lymphocytes were investigated under experimental conditions as described (23).

Analytical Procedures. Reversed-phase high-pressure liquid chromatography was used in the fractionation of acid-soluble extracts of cytolytic lymphocytes for determining the radioactivity present in AdoMet, AdoHcy, and deazaAdoHcy and for the quantitation (via ultraviolet absorbance) of deazaAdoHcy. Separations were accomplished on a Waters μBondapak C_{18} column (0.39 × 30 cm) mounted in a high-pressure liquid chromatograph from Instrumentation Specialties Co. This column was eluted with a linear (80-min) gradient, with 50 mM ammonium phosphate (pH 5.4) and 50 mM ammonium phosphate, pH 6.0/60% (vol/vol) acetonitrile as the two eluents. The column flow rate was 60 ml/hr, and a 16-min gradient delay was used. Under these chromatographic conditions (methionine plus homocysteine), AdoMet, deazaAdo, deazaAdoHcy, AdoHcy, adenosine, and 2-chloroadenosine were all completely resolved from each other with retention times of 3–4, 11.2, 24.7, 26.6, 29.1, 32.0, and 39.3 min, respectively. The column effluent was monitored at both 254 and 280 nm with full-scale absorbance ranges of 0.02 and 0.04, respectively. Fifty-microliter samples of each cell extract were injected into the liquid chromatograph. In those experiments involving [^{3}H]methionine-labeled cells, the effluent of the liquid chromatograph was collected at 1.0-min intervals, and these fractions were monitored in a liquid scintillation spectrometer. Peaks were identified by comparison with the retention times of authentic standards or by their A_{254}/A_{280} ratios or by both methods. Concentrations of 2-chloroadenosine and deazaAdoHcy present in cell extracts were calculated from response factors (ultraviolet peak area per nmol of compound), determined by injecting known amounts of authentic marker compounds into the liquid chromatograph. The amount of 2-chloroadenosine present in each extract was used to normalize each analysis (whether based upon radioactivity or ultraviolet peak area) to the original cell count.

cAMP present in acid-soluble extracts of cytolytic lymphocytes was determined by radioimmunoassay after purification of the cell extracts through sequential columns of aluminum oxide and Dowex 1-X8 and subsequent 2′-*O*-succinylation of samples (23).

Acid-soluble extracts of cytolytic lymphocytes were analyzed for ribonucleoside 5′-phosphates by anion-exchange high-pressure liquid chromatography (23).

RESULTS

Inhibition of LMC by DeazaAdo. As a continuation of our study of the effects of adenosine (17) and its structural analogs (23, 24) on LMC, deazaAdo was tested in the *in vitro* LMC assay and was found to be highly inhibitory (Fig. 1). The 50% inhibitory concentration (IC_{50}) for deazaAdo was 20 μM when this adenosine analog was added to the cytolytic lymphocytes at the start of the LMC assay. The inhibitory potency of deazaAdo was increased 2.5-fold (to IC_{50} = 8 μM) when the lymphocytes were preincubated for 60 min with this agent prior to the start of the LMC assay. Nevertheless, this inhibitory effect of deazaAdo was almost fully reversed when the lymphocytes were pretreated for 60 min with 20 μM deazaAdo and were then transferred quickly to drug-free medium for the start of the LMC assay. The activity of deazaAdo in the LMC assay was unaffected by addition of *erythro*-9-(2-hydroxy-3-nonyl)adenine (7.9 μM), a potent inhibitor of adenosine deaminase (EC 3.5.4.4) (25); this result is consistent with reports that deazaAdo is not a substrate for the deaminase (26, 27). Unlike adenosine and many of its structural analogs (23, 24), deazaAdo was not potentiated in its inhibition of LMC by Ro 20-1724 (50 μM), a powerful inhibitor of cAMP phosphodiesterase (28).

Recent reports have shown that deazaAdo is both an inhibitor and a substrate for adenosylhomocysteinase (EC 3.3.1.1) (27) and that Hcy enhanced both the elevation of AdoHcy and the inhibition of DNA methylation caused by adenosine in lymphoma cells (29). In light of these reports, Hcy was examined in the LMC assay. By itself, Hcy was completely without effect on cytolysis at concentrations as high as 1.0 mM. However, at concentrations as low as 25 μM, this amino acid clearly en-

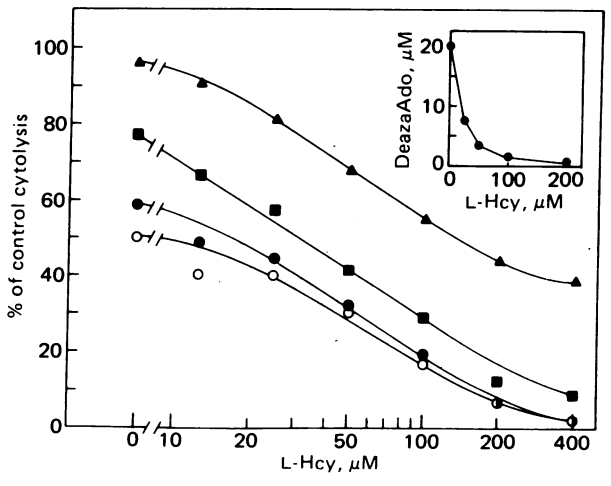

FIG. 1. Effect of deazaAdo and Hcy on lysis of ^{51}Cr-labeled EL4 cells by cytolytic lymphocytes. Each point represents the mean of duplicate assays. Results are expressed as the percent of ^{51}Cr released specifically by the drug-treated cells as compared with that released by the untreated (saline control) cells. DeazaAdo: ▲, 1.0 μM; ■, 5.0 μM; ●, 10 μM; ○, 20 μM. (*Inset*) Concentrations of deazaAdo and Hcy that, alone or in combination, resulted in 50% inhibition of LMC were estimated by replotting the data in Fig. 1 as percent of control cytolysis against deazaAdo concentration at several different fixed concentrations of Hcy.

hanced the LMC-inhibitory effect of deazaAdo (Fig. 1). This potentiation between deazaAdo and Hcy is readily apparent when the data in Fig. 1 are replotted in the form of an isobologram (Fig. 1, *inset*). The IC_{50} for deazaAdo was lowered from 20 to 0.4 μM by simultaneous addition of 200 μM Hcy to the LMC assay. Preincubation of cytolytic lymphocytes with varying concentrations of deazaAdo in the presence of Hcy (200 μM) for 60 min resulted in a further decrease in the IC_{50} to 0.2 μM. The inhibition of LMC caused by the combination of deazaAdo plus Hcy was slowly reversible: lymphocytes pretreated for 60 min with 5.0 μM deazaAdo plus 200 μM Hcy recovered approximately one-half of their cytolytic activity after 2.5 hr of incubation in drug-free medium.

Biochemical Effects of DeazaAdo in Cytolytic Lymphocytes. Because the inhibition of LMC by adenosine (17) and many of its structural analogs (23, 24) is associated with an increase in lymphocytic cAMP, deazaAdo was examined for its ability to elevate cAMP in cytolytic lymphocytes. In two separate experiments, deazaAdo, at concentrations from 40 to 150 μM, had little or no effect on cAMP levels. Neither Ro 20-1724 (50 μM) nor Hcy (200 μM) augmented the ability of deazaAdo to increase lymphocytic cAMP. In these same experiments, adenosine (10–40 μM) caused large increases in lymphocytic cAMP and was potentiated markedly in this activity by Ro 20-1724 (data not shown).

It was also of interest to learn whether deazaAdo was itself metabolized to 5′-nucleotides in cytolytic lymphocytes or whether this analog caused a decrease in the pool sizes of endogenous ribonucleoside 5′-triphosphates in the lymphocytes. Lymphocytes were therefore incubated for 60 min with saline, deazaAdo alone (40 μM), or deazaAdo (10 μM) plus Hcy (200 μM), and acid-soluble extracts of these cells were analyzed by anion-exchange high-pressure liquid chromatography (23). This procedure, which has been successful in demonstrating the metabolism of many other adenosine analogs to 5′-nucleotides in cytolytic lymphocytes (23, 24), failed to detect any nucleotide metabolites of deazaAdo. This result is supported by the recent finding that adenosine kinase (EC 2.7.1.20), highly purified from rabbit liver, does not use deazaAdo as a substrate (R. L. Miller, personal communication). Quantitative analysis of the amounts of CTP, UTP, ATP, and GTP present in these same cellular extracts showed that deazaAdo, either alone or in combination with Hcy, did not cause a decrease in the pool sizes of any of these endogenous ribonucleotides (data not shown).

In view of the inhibitory properties of deazaAdo towards adenosylhomocysteinase (27) and the consequent elevation of AdoHcy caused by deazaAdo in several types of mammalian cells (27, 30), it was of particular interest to determine the effect of deazaAdo upon AdoHcy levels in the cytolytic lymphocytes. However, direct measurements of AdoHcy in extracts of lymphocytes by reversed-phase high-pressure liquid chromatography proved unfeasible, due both to the small lymphocytic pool (<20 pmol/10^6 cells) of AdoHcy and to the paucity of tissue available for experimentation. For this reason, an indirect approach to this question was taken. The methionine pool of the lymphocytes was labeled with L-[2-^{3}H]methionine, and deazaAdo was then examined for its effect upon the flow of radioisotope along the pathway:

L-Methionine → AdoMet → AdoHcy → adenosine + L-homocysteine.

Extracts of cytolytic lymphocytes were fractionated by reversed-phase high-pressure liquid chromatography, and the cellular levels of radioactive AdoMet, AdoHcy, and deazaAdoHcy were determined. Extracts of control cells yielded only two prominent peaks of radioactivity, one corresponding to methionine/homocysteine and one coincident with AdoMet; little or no radioactivity was eluted at the retention time for AdoHcy. By contrast, extracts of deazaAdo-treated cells yielded two additional peaks of radioactivity, which were eluted with the same retention times as deazaAdoHcy and AdoHcy.

By this method it was possible to show that 40 μM deazaAdo caused a rapid increase in the level of [^{3}H]AdoHcy in cytolytic lymphocytes that had been labeled with [2-^{3}H]methionine (Fig. 2*B*). After 30 min of drug treatment, the lymphocytic content of [^{3}H]AdoHcy was elevated 48-fold over that of control cells. When the lymphocytes were washed and transferred to drug-free medium after 30 min of treatment of 40 μM deazaAdo, the cellular accumulation of [^{3}H]AdoHcy was dissipated rapidly (Fig. 2*B*). The cellular level of [^{3}H]AdoMet declined throughout the course of this experiment (Fig. 2*A*); however, treatment of the lymphocytes for 30 min with deazaAdo caused an elevation of [^{3}H]AdoMet of approximately 2-fold relative to control cells. The levels of [^{3}H]AdoHcy and [^{3}H]deazaAdoHcy found in the extracts of lymphocytes in this experiment, although demonstrable by radioactivity measurements (Fig. 2), were undetectable by ultraviolet (254 and 280 nm) monitoring of the eluate from the reversed-phase column. On the basis of the empirical sensitivity of this instrument (approximately 50 pmol of either AdoHcy or deazaAdoHcy), it can be estimated that the cellular levels of AdoHcy and deazaAdoHcy depicted by radioactivity in Fig. 2 are less than 20 pmol/10^6 cells. The elevation of lymphocytic [^{3}H]AdoHcy and [^{3}H]AdoMet by deazaAdo was dependent upon the concentration of drug added to the cells (Fig. 3). The cellular accumulation of [^{3}H]deazaAdoHcy was maximal with 10 μM deazaAdo.

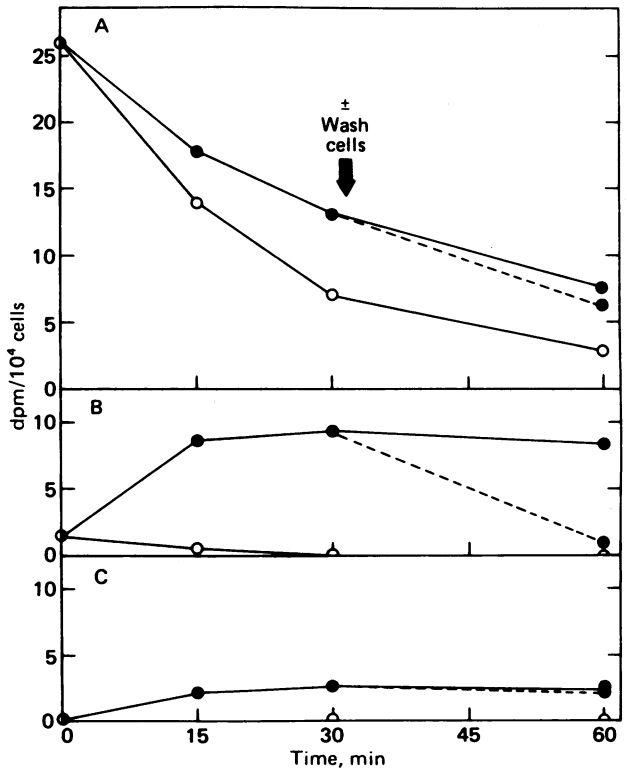

FIG. 2. Time course of effect of deazaAdo on metabolism of L-[2-^{3}H]methionine in cytolytic lymphocytes. Lymphocytes were labeled with L-[2-^{3}H]methionine, washed, resuspended in fresh medium, and incubated at 37°C with (●) or without (○) 40 μM deazaAdo. After 30 min of drug treatment, some cellular suspensions (dashed lines) were centrifuged and the cells were washed rapidly and resuspended in drug-free medium for an additional 30 min of incubation at 37°C. Incubations were terminated at the indicated times and the amounts of radioactivity present in (*A*) AdoMet, (*B*) AdoHcy, and (*C*) deazaAdoHcy in each cell extract were determined.

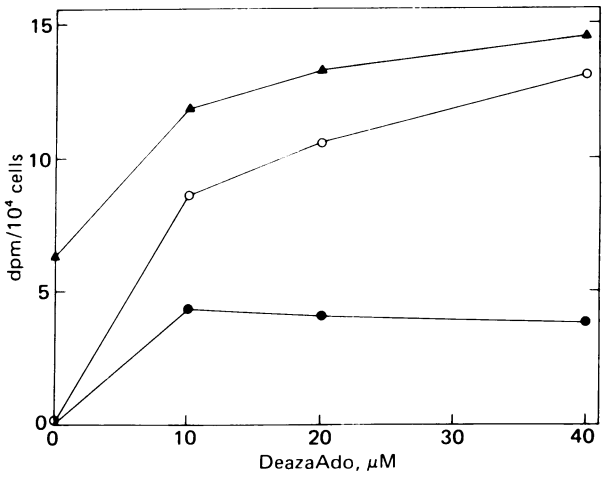

FIG. 3. Effect of deazaAdo concentration on levels of radioactive AdoMet (▲), AdoHcy (○), and deazaAdoHcy (●) in cytolytic lymphocytes that had been labeled with L-[2-^{3}H]methionine. Cells were treated with deazaAdo for 60 min.

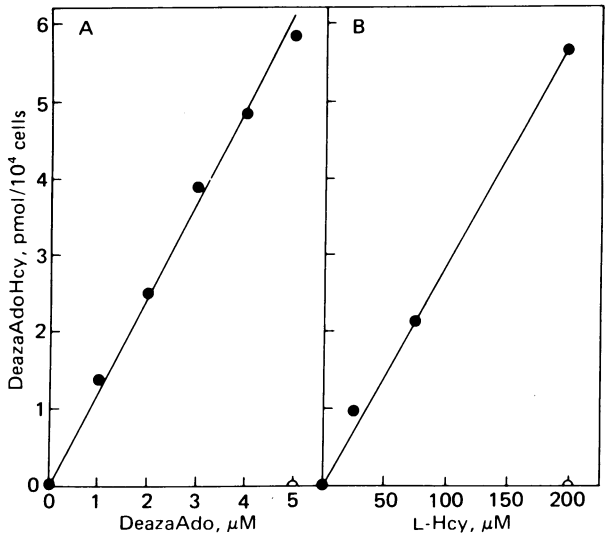

FIG. 4. Dependence of deazaAdoHcy formation in cytolytic lymphocytes on concentrations of deazaAdo and Hcy. Lymphocytes were incubated for 60 min with (*A*) different concentrations of deazaAdo in the presence of saline (○) or 200 μM Hcy (●) and with (*B*) different concentrations of Hcy in the presence of saline (○) or 5.0 μM deazaAdo (●).

Incubation of cytolytic lymphocytes with deazaAdo *plus* Hcy resulted in the metabolic formation of large amounts of deazaAdoHcy; extracts of these cells yielded a prominent peak of deazaAdoHcy in the ultraviolet absorbance tracings of the eluate from the reversed-phase column. The lymphocytic buildup of deazaAdoHcy was dependent upon the concentrations of both deazaAdo (Fig. 4*A*) and Hcy (Fig. 4*B*) in the cellular incubation medium. After 60-min incubations with 5.0 μM deazaAdo plus 200 μM Hcy, the lymphocytes contained 560–580 pmol of deazaAdoHcy per 10^6 cells; this cellular level of deazaAdoHcy is as great as the lymphocytic content of ATP (23). Lymphocytes containing deazaAdoHcy (due to prior incubation with deazaAdo plus Hcy) eliminated this metabolite slowly after transfer of the cells to drug-free medium. After 1.0 and 2.5 hr in drug-free medium, the lymphocytes still contained 173 and 49 pmol, respectively, of deazaAdoHcy per 10^6 cells as compared with a starting level of 458 pmol/10^6 cells. Cells preloaded with deazaAdoHcy but then incubated for 2.5 hr in the original drug-containing medium also exhibited a progressive but slower decrease in deazaAdoHcy (to 263 pmol/10^6 cells). This latter effect may be due to metabolism of L-homocysteine and consequent reversal of the adenosylhomocysteinase reaction responsible for the synthesis of deazaAdoHcy.

DISCUSSION

Two principal findings have emerged from the present studies: (*i*) 3-Deazaadenosine appears to be a highly specific antimetabolite whose effect upon cells is limited to elevation of AdoHcy or deazaAdoHcy or both and consequent inhibition of AdoMet-utilizing methyltransferases; (*ii*) an unidentified AdoMet-utilizing methylation reaction appears to play a crucial role in the yet unknown mechanism of LMC.

The only metabolic fate known for deazaAdo is conversion to deazaAdoHcy via adenosylhomocysteinase (27, 30); this enzyme catalyzes a reaction that is readily reversible and that, in fact, favors synthesis of AdoHcy (from adenosine and L-homocysteine) over hydrolysis (31). No evidence of nucleotide formation from deazaAdo could be found in the present studies, and deazaAdo did not perturb the levels of endogenous ribonucleoside 5′-triphosphates in the cytolytic lymphocytes. Moreover, unlike adenosine (17) and many of its other structural analogs (23, 24), deazaAdo does not cause an appreciable elevation of cAMP in the lymphocytes; presumably, deazaAdo does not bind productively to the adenosine receptor present on these cells (23). As both a substrate and an inhibitor of adenosylhomocysteinase (27, 30), deazaAdo undergoes metabolism to deazaAdoHcy and inhibits the catabolism of metabolically produced AdoHcy. Both AdoHcy (ref. 32 and refs. cited therein) and deazaAdoHcy (33) are potent inhibitors of a number of different AdoMet-utilizing methyltransferases. Hence, the cellular buildup of AdoHcy or deazaAdoHcy or both caused by deazaAdo would be expected to result in inhibition of many of the methyltransferase reactions that use AdoMet as methyl donor. Recently, it has been shown that elevation of AdoHcy in lymphoid cells treated with adenosine plus Hcy resulted in inhibition of methylation of DNA (29), RNA (34), and protein (35).

The effects of deazaAdo on cytolytic lymphocytes indicate that these cells require the participation of a methylation reaction in the performance of their cytolytic function. Concentrations of deazaAdo that inhibit LMC also cause a buildup of AdoHcy and deazaAdoHcy in the lymphocytes. Hcy both potentiates the inhibition of LMC by deazaAdo and augments greatly the cellular buildup of deazaAdoHcy. A qualitative correlation exists between the extent and rapidity of reversal of deazaAdo inhibition of LMC and the ability of cytolytic lymphocytes to eliminate the AdoHcy or deazaAdoHcy accumulated during the drug treatment. The concurrent elevation of lymphocytic AdoMet caused by deazaAdo is attributed to diminished utilization of AdoMet by methyltransferases whose activities are inhibited by elevated cellular levels of AdoHcy or deazaAdoHcy or both; this effect of deazaAdo on AdoMet levels in cytolytic lymphocytes has been observed in other tissues (27, 30). Finally, the apparent lack of effect of deazaAdo on all aspects of lymphocytic nucleotide metabolism investigated in this report accentuates these positive findings concerning AdoHcy metabolism.

Whereas the effects of deazaAdo on cytolytic lymphocytes appear to be uncomplicated, those of adenosine appear to be complex. This latter agent inhibits LMC, causes a marked elevation of lymphocytic cAMP, and is potentiated in both activities by Ro 20-1724 (17, 24). In addition, adenosine causes an elevation of AdoHcy in cytolytic lymphocytes, and both the inhibiton of LMC and the elevation of lymphocytic AdoHcy are potentiated by Hcy (unpublished data). Thus, it is unclear

at present whether adenosine inhibits LMC by two discrete and concurrent mechanisms—one involving an elevation of cAMP and the other involving an elevation of AdoHcy in cytolytic lymphocytes—or whether these two biochemical phenomena are coordinately interrelated. It is possible that the cAMP-mediated inhibition of LMC is effected either by direct modulation of the activity of a methyltransferase or by regulation of the AdoMet/AdoHcy metabolism in the lymphocytes. Indeed, given the variety of molecular species (DNA, RNA, proteins, carbohydrates, phospholipids, etc.) that serve as substrates for different methyltransferases *in vivo*, it is tempting to speculate that the pleiotypic effects of cAMP (36) may be realized in part through a generalized regulation of AdoMet-utilizing methyltransferases, possibly via cellular levels of AdoMet or AdoHcy or both.

Little can be said at present concerning the identity or biochemical function of the methylated substance(s) whose continuous formation in cytolytic lymphocytes seems to be essential to the process of LMC. Other specialized cellular functions, including the chemotaxis of neutrophils (37, 38) and of monocytes (35), the aggregation of platelets (39), and exocytotic secretion (40), appear to require methylation of carboxyl side chains of cellular proteins. Perhaps cytolytic lymphocytes must secrete a toxic material into the target cell in order to effect lysis (2) and protein carboxymethylation is required for such secretion to take place. Alternatively, charge neutralization via carboxymethylation may allow the lymphocytes to transform a peptide into a more hydrophobic entity for insertion into the membrane of the target cell to cause channel formation (21).

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