Selective Adenosine Release from Human B but Not T Lymphoid Cell Line*

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Intracellular adenosine formation and release to extracellular space was studied in WI-L2-B and SupT1-T lymphoblasts under conditions which induce or do not induce ATP catabolism. Under induced conditions, B lymphoblasts but not T lymphoblasts, release significant amounts of adenosine, which are markedly elevated by adenosine deaminase inhibitors. In T lymphoblasts, under induced conditions, only simultaneous inhibition of both adenosine deaminase activity and adenosine kinase activities resulted in small amounts of adenosine release. Under noninduced conditions, neither B nor T lymphoblasts release adenosine, even in the presence of both adenosine deaminase or adenosine kinase inhibitors. Comparison of B and T cell's enzyme activities involved in adenosine metabolism showed similar activity of AMP deaminase, but the activities of AMP-5'-nucleotidase, adenosine kinase and adenosine deaminase differ significantly. B lymphoblasts release adenosine because of their combination of enzyme activities which produce or utilize adenosine (high AMP-5'-nucleotidase and relatively low adenosine kinase and adenosine deaminase activities). Accelerated ATP degradation in B lymphoblasts proceeds not only via AMP deamination, but also via AMP dephosphorylation into adenosine but its less efficient intracellular utilization results in the release of adenosine from these cells. In contrast, T lymphoblasts release far less adenosine, because they contain relatively low AMP-5'-nucleotidase and high adenosine kinase and adenosine deaminase activities. In T lymphoblasts, AMP formed during ATP degradation is not readily dephosphorylated to adenosine but mainly deaminated to IMP by AMP deaminase. Any adenosine formed intracellularly in T lymphoblasts is likely to be efficiently salvaged back to AMP by an active adenosine kinase. In general, these results suggest that adenosine released is produced only by active cells (adenosine producers) whereas other cells with enzyme combination similar to SupT1-T lymphoblasts cannot produce significant amounts of adenosine even in stress conditions.

Adenosine is an important autacoid which regulates cellular functions through its attachment to extracellular adenosine receptors (1). Adenosine has already been well established as having pharmacological effects in cardiovascular, neurologic, and immune systems (2), but the mechanism of adenosine release from cells is still not clear. Therefore, understanding how adenosine is produced by different cells is important because of its role in intercellular communications.

In severe combined immunodeficiency disease, associated with adenosine deaminase (ADA) deficiency (3-6), depressed ADA activity results not only in massive accumulation of intracellular dATP and extracellular deoxyadenosine but also causes a significant increase of adenosine levels in human plasma (7). The accumulation of deoxyadenosine in ADA deficiency is caused by an inhibition of the catabolic pathway of dATP which proceeds exclusively via ADA reaction (8). However, the accumulation of adenosine remains unclear because it has been described that ATP catabolism proceeds mainly via AMP deamination (8).

In lymphocytes, adenosine acts via adenosine receptors, which have been found on the surface of T cells (9) and B cells (10). Adenosine can inhibit T and B cell growth and can lead to cell death, especially of T cells. In the immune system, adenosine also inhibits monocyte maturation, blastogenesis of peripheral and spleen lymphocytes and of thymocytes, production of immunoglobulins, and expression of T lymphocyte cell surface receptors (11-13). Inhibition of ADA activity by the specific inhibitors, 2'-deoxycoformycin or erythro-9-(2-hydroxy-3-nonyl)adenine, significantly enhances the toxicity of adenosine to the immune system (14).

The mechanism of adenosine formation and release from cells can differ in different tissues because adenosine can be formed both intracellularly (15) and extracellularly (16), as well as from different precursors such as ATP, S-adenosyl homocysteine, and cAMP. However, extracellular or intracellular ATP seems to be the major source for adenosine formation, especially during physiological stress, when intracellular ATP degradation is induced (17).

It has recently been shown that B lymphocytes are capable of actively degrading extracellular ATP to adenosine, presumably because of their active ectoATPase, ectoADPase, and ectoAMPase (ecto-5'-nucleotidase) activities (18). In contrast, T lymphocytes showed no or at best little activities of these ectonucleotidases, indicating that B lymphoblasts can produce adenosine extracellularly more efficiently than T lymphoblasts.

Our previous studies on intracellular ATP catabolism in T cells, suggested that ATP degradation in these cells proceeds almost exclusively via AMP deamination. There was no extracellular accumulation of adenosine in the presence of the ADA inhibitor, deoxycoformycin, in normal noninduced conditions, and very little when ATP degradation was accelerated (8, 19). We concluded that no significant levels of adenosine were formed from ATP in T lymphocytes. However, it is unclear whether B cells, which are active in extracellular ATP...
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degradation, can produce adenosine from intracellular ATP, especially when ATP degradation is accelerated.

In this paper, intracellular ATP degradation in WI-L2-B lymphoblasts and Sup-T1-T lymphoblasts is compared under conditions when ATP degradation is induced or noninduced and under conditions when adenosine deaminase or adenosine kinase or both are inhibited. Increased adenosine production by B lymphoblasts as compared to T lymphoblasts is demonstrated. The mechanism for the increased adenosine production by B lymphoblasts is evaluated.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—[8-14C]Adenosine (56 mCi/mmol), [8-14C]adenine (56 mCi/mmol), and [8-14C]AMP were (45 mCi/mmol) purchased from Moravek Biochemicals, Inc., Brea, CA. Nonradioactive purine nucleotides, nucleosides and bases, 2'-deoxycoformycin, 2-deoxy-d-glucose, and alamine (tri-n-octylamine) were purchased from Sigma. 5-Iodotubercidin was purchased from RBI Research Biochemicals, Natick, MA. Kodak 135X-4 cellulose thin layer chromatography (TLC) sheets were obtained from Eastman Kodak. Polyethylenimine-cellulose TLC sheets were obtained from Brinkman Instruments, Inc., Westbury, NY. Freon (trichlorotrifluoroethane) was purchased from BOC Gases, Inc., Cranbury, NJ. Fetal bovine serum was obtained from Gemini Products, Calabasas, CA.

**Cells**—Two cell lines, human B (WI-L2) and T (Sup-T1) lymphoblastoid cells (20, 21) (obtained from Dr. J. E. Seegmiller, Department of Medicine, University of California, San Diego), were used in these studies. The WI-L2, an Epstein-Barr virus-transformed cell, secretes IgG and IgM and represents a stage close to maturity. The Sup-T1 cells are 21% CAII + cells, express T3, and have the enzymatic activities of T lymphoblasts.

- **Preparation of Cells**—WI-L2 and Sup-T1-T lymphoblasts were suspended in the growth medium containing cellular levels of radioactive nucleosides and bases were analyzed as follows. Prior to inducing ATP catabolism, the nonincorporated adenine was washed out with 200 μl of 0.4 M perchloric acid, left on ice for 5 min and then neutralized with 100 μl of alamine/freon mixture (2/8, v/v) (24). Separate purine nucleotides extracted from cells were separated on polyethyleneimine-cellulose using a 20 μl acetic acid buffer gradient (0.5, 2, 4 M) according to Henderson et al. (23).

**Measurements of Enzyme Activities**—Purine enzymes, AMP deaminase, AMP-5'-nucleotidase, AK, and ADA were assayed radiochemically in intact cells using 8-14C-labeled substrates.

- **Preparation of Cells**—WI-L2 and Sup-T1-T lymphoblasts were suspended in the presence or absence of 20 μM 2'-deoxycoformycin (25, 26) for 5 h. Production and release of ATP catabolates were measured in the culture medium.

**TABLE I**

<table>
<thead>
<tr>
<th>ATP catabolates released from non-stressed human B and T lymphoblasts</th>
<th>h</th>
<th>Azo Ino Hyp</th>
<th>Azo Ino Hyp</th>
</tr>
</thead>
<tbody>
<tr>
<td>B lymphoblasts</td>
<td>0</td>
<td>ND*</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>ND</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>ND</td>
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<td>ND</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>ND</td>
<td>41</td>
</tr>
</tbody>
</table>

**T lymphoblasts**

- **Preparation of Cells**—WI-L2 and Sup-T1-T lymphoblasts were suspended in the presence or absence of 20 μM 2'-deoxycoformycin for 5 h. Production and release of ATP catabolates were measured in the culture medium.

*ND, nondetected.*
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**FIG. 1.** Induced ATP degradation in human B and T lymphoblasts. Human B (A and B) or T (C and D) lymphoblasts (10^6 cells/ml) were preincubated with radioactive adenine (10 μCi) for 60 min and then with (B and D) or without (A and C) 20 μM 2'-deoxycoformycin. Cells were then resuspended in glucose-free medium and incubated with 5.5 mM 2-deoxyglucose for 60 min. At indicated times, 10^6 cells were analyzed by TLC to determine radiolabeling of nucleotides, and 9 X 10^6 cells were analyzed by HPLC to determine amounts of intracellular nucleotides.

To determine the apparent activity of AMP deaminase, cells (10^6) were incubated in the presence or absence of 20 μM 2'-deoxycoformycin with 2 μCi of [8-^14C]adenine (0.1 mM) for 60 min and the flow of radioactivity via the AMP deamination reaction (apparent activity) was measured as the summation of the counts/min of IMP + XMP + GMP + GDP + GTP + Hyp + Ino + Xao + Gua + Guo.

To evaluate the apparent activity of AMP-5'-nucleotidase, cells (10^6) were incubated with 2 μCi of [8-^14C]adenine (0.1 mM) in the presence or 20 μM 2'-deoxycoformycin, and the flow of radioactivity via the AMP dephosphorylation reaction was measured as the production of Ado.

To evaluate the apparent activity of ADA, cells (10^6) were incubated in the presence of the AK inhibitor 1 μM 5-iodotubercidin with 2 μCi of [8-^14C]adenosine (1.5 mM) and the flow of radioactivity via the adenosine deamination reaction was measured as the summation of the counts/min of Ino + Hyp + IMP.

To evaluate the apparent activity of AK, cells (10^6) were incubated in the presence of 20 μM 2'-deoxycoformycin with 2 μCi of radioactive [8-^14C]adenosine (1.5 mM) and the flow of radioactivity via the adenosine phosphorylation reaction was measured as the summation of the counts/min of AMP + ADP + ATP.

HPLC Determination of Purine Metabolite Levels—HPLC determination of purine nucleotides, nucleosides, and bases was done using a Waters System containing two 510 pumps, static mixing chamber, WISP 712 automatic sample injector with refrigeration. For nucleotide analysis a 441Z UV detector set for 254 nm with Waters 820 software installed in an APC IV computer was used. Nucleotides were separated using an Altex Ultrasil-AX 10 μm (250 X 4.6 mm) column and a Brownlee Anion AX-GU, 10 μm (30 X 46 mm) guard column. A gradient of buffers (20 mM KH₂PO₄, pH 3.85, to 0.25 M KH₂PO₄, 0.6 M KCl, pH 3.45) was used with a flow rate of 1 ml/min. Additionally, for analysis of nucleosides and bases 990 photodiode array detector and 680 automated gradient controller, 990 plotter, and Waters 990 software were used. Purine nucleosides and bases were separated using a Waters μBondapak C-18 column (3.9 X 300 mm) and Brownlee RP-18 guard column (15 X 3.2 mm) with a Newgard holder. A gradient of buffers 20 mM phosphoric acid, pH 3.5, to 5% (acetoniitrile) was used.

Each experiment was repeated at least three times and typical results are presented. Average deviation of presented results from the mean values was less than 10%.

**RESULTS AND DISCUSSION**

ATP catabolism was studied in the WI-L2 B and SupT1-T lymphoid cell lines under noninduced conditions (without any
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FIG. 2. Release of purine nucleosides and bases from cells undergoing induced ATP degradation. Human B (A and B) or T (C and D) lymphoblasts (10^7 cells/ml) were preincubated with 10 μCi of radioactive adenine to label intracellular ATP. After washing out unused adenine, cells (10^6 cells/0.1 ml) were incubated with 5.5 mM 2-deoxyglucose in glucose-free growth medium, with (B and D) or without (A and C) 20 μM 2'-deoxycoformycin. Radioactive adenosine, inosine, and hypoxanthine were measured in the incubation medium.

metabolic stressing additions to the growth media) and under induced conditions (following addition of 2-deoxyglucose or sodium azide to glucose-deficient growth medium). When degradation of radioactive ATP was studied, cells were preincubated with radioactive adenine. Labeling of ATP with radioactive adenine results in almost exclusive radiolabeling of ATP (results not shown). Other adenine nucleotides, ADP and AMP, contained less than 10% of the total nucleotide radioactivity and IMP and guanine nucleotides together represent less than 5%. There was no significant difference in the initial level of radiolabeled ATP in B and T cells and preincubation with radioactive adenine did not change practically intracellular levels of adenosine nucleotides.

As shown in Table I, under noninduced conditions, during up to 5 h of incubation, there was no detectable release of adenosine in cells with active or inhibited ADA (without or with deoxycoformycin) in either B or T lymphoblasts. Only small amounts of inosine or hypoxanthine were found in the medium and these results agree with observations previously described for T cells (8). This may indicate that ATP degradation under normal, noninduced conditions is balanced by ATP conservation reactions.

When ATP catabolism was induced by 2-deoxyglucose or (sodium azide) in the absence of glucose, ATP degradation to AMP seems to be similar in B and T lymphoblasts (Fig. 1). In 10 min, about 70% of ATP is degraded in both B and T lymphoblasts and no changes in ADP levels was observed. AMP level somewhat increased to reach maximum at 10–20 min incubation with 2-deoxyglucose and decrease later (Fig. 1). The same results were obtained when concentrations of metabolites were measured or radioactivity of metabolites was monitored. Marked differences were, however, found between these two cell lines in the formulation of nucleosides and bases, the end products of ATP degradation. In B cells, when the net ATP degradation is accelerated by 2-deoxyglucose, hypoxanthine and inosine were the major products of ATP catabolism, with clearly detectable levels of adenosine released from the cells (Fig. 2A). In the presence of 20 μM 2'-deoxycoformycin, which completely and specifically inhibits ADA (25), induction of ATP catabolism in B cells results in a substantial increase in the release of adenosine in to the medium (Fig. 2B). A concentration of 20 μM 2'-deoxycoformycin does not effect AMP deaminase activity: 26,132 cpm/10^6 cells in the absence and 25,592 cpm/10^6 cells in the presence of 20 μM 2'-deoxycoformycin passed through the AMP deamination reaction. Simultaneously there is a decrease of the hypoxanthine level with no change in the level of inosine compared to cells without deoxycoformycin formed.
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**Table II**

**Effect of 2-deoxyglucose on adenosine kinase activity in human B and T lymphoblasts**

Lymphoblasts (B or T) (10^7 cells/ml) were preincubated with 20 μM 2'-deoxycoformycin for 20 min and then incubated with 2 μCi of radioactive adenosine in the presence or absence of 5.5 mM 2-deoxyglucose. Activity of adenosine kinase in intact cells was measured as the sum of AMP + IMP + ADP + ATP + Ino + Hyp.

**Table III**

**Effect of 5-iodotubercidin on adenosine formation**

Cells (human B or T lymphoblasts, 10^7 cells/ml) were incubated with 2 μCi of adenosine to label intracellular ATP. Cells were then incubated in glucose free medium with 20 μM 2'-deoxycoformycin, 5.5 mM 2'-deoxyglucose, and with or without 1 μM 5-iodotubercidin.

**Table IV**

**Activity of purine enzymes in cell extracts and in intact B and T lymphoblasts**

Purine enzymes were assayed radiochemically in cell extracts and in intact cells.

**Table V**

**Purine compounds released from human peripheral blood lymphocytes**

Human peripheral blood lymphocytes (10^7) were incubated with 5.5 mM 2-deoxyglucose in the presence of 20 μM 2'-deoxycoformycin for 60 min. After incubation purine compounds were determined by HPLC.
intact cells or from intact cells to extracts is often difficult (27), this study showed good correlation between enzyme activities in these two systems. This combination of activities, high AK and low AMP-5'-nucleotidase, is consistent with the only form from extracellular ATP as it has been shown before (18) but also from the intracellular pool of ATP. Therefore, in ADA deficiency it is possible that B cells contribute more than T cells to elevated adenosine levels.

These results also indicate that under stress conditions, when net ATP catabolism is enhanced, other cell types with a combination of enzyme activities similar to B cells (high 5'-nucleotidase and low AK and ADA) can produce and release adenosine.

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REFERENCES