Induction of differentiation in leukemia THP-1 cells treated with adenosine and ATP

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Abstract: Adenosine and ATP have been implicated in the regulation of inflammatory responses. Furthermore, adenosine and ATP affect cell growth, cell differentiation, and apoptosis. The aim of this study was to evaluate differentiation inducing abilities of adenosine and ATP on human monocytic leukemia THP-1 cells. Adenosine at 10⁻³ M markedly induced the differentiation in leukemia THP-1 cells as assessed by evaluating the expression of CD11b. Similarly, ATP at 10⁻³ M significantly induced the differentiation in THP-1 cells. Simultaneously, adenosine at 10⁻² M and ATP at 10⁻³ M significantly inhibited the cell number in THP-1 cells. Next, we have evaluated abilities of adenosine and ATP on THP-1 cells differentiated by differentiation inducers all-trans retinoic acid (ATRA), 1,25-dihydroxy-vitamin D₃ (VD₃) and phorbol 12-myristate 13-acetate (PMA). Interestingly, adenosine at 10⁻³ M significantly potentiated ATRA-induced CD11b expression. In addition, ATRA significantly increased adenosine-induced CD11b expression. Similarly, ATP at 10⁻³ M significantly potentiated ATRA-induced CD11b expression. In addition, ATRA potentiated ATP-induced CD11b expression. In contrast, VD₃ and PMA further increased the expression of CD11b in the presence of adenosine of ATP, respectively. These results suggest that adenosine and ATP may induce the differentiation in human leukemia THP-1 cells, and may further increase the ATRA-induced differentiation in THP-1 cells.

Key words: adenosine, ATP, LPS, differentiation, CD11b

Introduction

Regulation of hematopoietic cell differentiation has been studied using a variety of lineage specific leukemia cell lines. Human leukemia cell line THP-1 possesses immature promonocytic characteristics and proliferates continuously in the absence of growth factor. THP-1 cells differentiate into monocyte/macrophage by various inducers such as all-trans retinoic acid (ATRA), 1,25-dihydroxy-vitamin D₃ (VD₃) and phorbol 12-myristate 13-acetate (PMA)⁴⁻⁵. ATRA and VD₃ can also induce monocytic differentiation of other leukemia cell lines⁶⁻⁸. ATRA is used to treat patients with acute promyelocytic leukemia (APL) and induces granulocytic differentiation of APL cells as well as several myeloid leukemia cell lines¹⁻⁴. In contrast, PMA induces macrophage differentiation of other leukemia cell lines⁶⁻⁸.

ATP enhanced IL-6-induced differentiation of mouse myelomonocytic leukemia M1 cells to macrophages, without inducing differentiation of M1 cells in the absence of IL-6¹¹. In addition, ATP affects cell growth of epidermoid carcinoma cells¹² and inhibit terminal differentiation of human keratinocytes¹³. Importantly, concentration requirement for ATP in normal tissue culture medium can exceed millimolar values¹⁴,¹⁵. However, it has been shown that extracellular ATP is not stable, and ATP is readily degraded by ectonucleotidases to adenosine. Adenosine is a purine nucleotide that is released from a variety of cells in response to

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metabolic stress or from the sympathetic nervous system. In addition, the accumulation of extracellular adenosine in inflamed and damaged tissues\(^{16-20}\) and the immunosuppressive properties of cAMP-elevating adenosine receptors indicate that signaling by A2a adenosine receptor (A2aR) on immune cells is a possible natural mechanism of inhibition and/or termination inflammation\(^{21-28}\). However, there is little information about the ability of adenosine on the differentiation of leukemia cells. It was useful and meaningful to gain information about possible abilities of adenosine and ATP on differentiation of leukemia cells. In this study, we have evaluated whether adenosine and ATP induced differentiation in human monocytic leukemia THP-1 cells. Here we show that adenosine and ATP induced differentiation in leukemia THP-1 cells. To gain information on the combination of differentiation inducers and adenosine or ATP, furthermore, we have simultaneously evaluated the abilities of adenosine and ATP on differentiation inducers-induced differentiation in THP-1 cells.

Materials and Methods

1. Materials

Adenosine, ATRA, ATP and PMA were provided by Sigma (St. Louis, MO). Phycoerythrin (PE)-conjugated human antibody against CD11b was purchased from BD Biosciences (San Diego, CA). VD3 was supplied by Calbiochem (San Diego, CA).

2. Cell Culture

Human leukemia cell line THP-1 (Riken Gene Bank, Tokyo, Japan) was maintained in RPMI-1640 supplemented with 10% FBS, 100 units/mL of penicillin and 100 µg/mL of streptomycin.

3. Assessment of differentiation

Cells (5×10\(^5\)) were incubated for 72 h. After washed with PBS, cells were incubated with PE-conjugated monoclonal antibody against CD11b for 30 min at 4°C in the dark. Cells were then washed with PBS. Finally, stained cells were analyzed on a flow cytometry (BEC-TON DICKINSON). Data are expressed as calculated by the CellQuest\(^\text{®}\) software.

4. Assessment of cell number

Cell number was evaluated by CellTiter 96\(^\text{®}\) AQueous One Solution Cell Proliferation Assay (Promega Corporation; Madison, WI). Briefly, cells (6×10\(^5\)) were incubated in 96-well culture plates for 72 h. After incubation, MTS solution were added to culture plates. The absorbance at a wavelength of 490 nm was measured at 2 h after the addition of MTS solution, and reference absorbance is 690 nm.

5. Assessment of apoptosis

Determination of apoptosis was evaluated as previously described\(^25\). Briefly, cells (6×10\(^4\)) were incubated in 96-well culture plates for 72 h. Cells were centrifuged and cell pellets were lysed. For this assay, 20 µL lysate was used. The lysate was added to streptavidine-coated 96-well plates, to which was added a mixture of biotinylated anti-histone- and peroxidase-coupled anti-DNA antibodies. Following a 2 h incubation, the amount of cytoplasmic nucleosome was quantified by the peroxidase retained in the immunocomplex, which was determined spectrophotometrically with 2,2'-azino-di[3-ethylbenzothiazolin-sulfonat] as substrate at an absorbance of 405 nm and reference absorbance is 492 nm.

6. Statistical analysis

Results were all expressed as the mean±SE. Statistical analysis was determined by one-way ANOVA for non-repeated to detect differences between multiple groups. Differences between groups were determined by Student-Newman-Keuls tets. Differences were considered to be significant when the P value was <0.05.

Result

1. Adenosine and ATP induced differentiation in U937 cells

CD11b is an early cell surface differentiation marker for mature myeloid and monocytic cells. Undifferentiated THP-1 cells do not express CD11b, whereas differentiated monocytic THP-1 cells express high levels of CD11b. THP-1 cells were treated with or without adenosine or ATP, and analyzed for CD11b expression by flow cytometry. Figure 1 shows the flow cytometric histograms and the emergence of the induced CD11b positive cells at 72 h after incubation. Adenosine at 10\(^{-3}\)M and ATP at 10\(^{-3}\)M markedly increased the expression of CD11b. Figure 1 also indicates that differentiation inducers ATRA, VD3 and PMA all increased the expression of CD11b.

To determine whether adenosine and ATP affect cell proliferation in THP-1 cells, THP-1 cells were treated for 72 h, and MTS solution was added over the last 2 h. Adenosine at 10\(^{-3}\)M significantly inhibited cell number (Fig. 2a).
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Similiarly, ATP at $10^{-3}$ M significantly inhibited cell number (Fig. 2b).

To exclude the possibility that anti-proliferative effects by adenosine and ATP were caused by the induction of apoptosis, we detected apoptosis as assessed by a cell death ELISA assay that detects cytosolic histone-bound DNA fragments formed in cells undergoing apoptosis. However, neither adenosine nor ATP at concentrations of up to $10^{-3}$ M induce nucleosomal DNA fragmentation in THP-1 cells (data not shown).

2. Abilities of adenosine and ATP on differentiation inducers-induced differentiation in U937 cells

The effects of combinations of differentiation inducers and adenosine or ATP on the expression of CD11b were studied. Figure 3a shows the flow cytometric histograms of CD11b positive cells treated with adenosine or ATP in the presence of each differentiation inducer ATRA, VD$_3$ and PMA. Figure 3b shows the comparisons of Geo Mean fluorescence intensity of three different experiments. Interestingly, adenosine at $10^{-3}$ M significantly potentiated ATRA-induced CD11b expression. In addition, ATRA significantly increased adenosine-induced CD11b expression. Similarly, ATP at $10^{-3}$ M significantly potentiated ATRA-induced CD11b expression. In addition, ATRA potentiated ATP-induced CD11b expression. In case of VD$_3$ and PMA, in contrast, VD$_3$ and PMA further increased the expression of CD11b in the presence of adenosine or ATP, respectively. However, neither VD$_3$ combined with adenosine or ATP, nor PMA combined with adenosine or ATP increase the expression of CD11b in THP-1 cells compared with VD$_3$ alone or PMA alone, respectively.

To determine the effects of combinations of differentiation inducers and adenosine or ATP on cell proliferation, we analyzed cell number in THP-1 cells as assessed by MTS assay.
Fig. 2 Effects of adenosine and ATP on cell number in leukemia THP-1 cells. Cells were incubated with adenosine or ATP for 72 h. Cell number was determined by colorimetric MTS assay. The data from three different specimens are shown. **P<0.01, when compared with untreated cells.

ATRA further inhibited adenosine at 10^{-3} M-inhibited cell number, without affecting ATP at 10^{-3} M-inhibited cell number (Fig. 4). In addition, VD3 further inhibited adenosine at 10^{-3} M-inhibited cell number, whereas VD3 prevented ATP at 10^{-3} M-inhibited cell number. Furthermore, PMA prevented ATP at 10^{-3} M-inhibited cell number, without affecting adenosine at 10^{-3} M-inhibited cell number.

Discussion

The results described in this paper indicate that adenosine and ATP induce the differentiation of monocytic leukemia THP-1 cells to mature monocyte/macrophage by examining differentiation marker CD11b. These abilities have directed attention to differentiation therapy against leukemia. In previous studies, some of the analogs of purines and pyrimidines can induce the differentiation of leukemia cells and inhibit the growth of tumor^{31-34}. These data also indicate that analogs of purines and pyrimidines cells are useful in anticancer chemotherapy including leukemia. As discussed in the Introduction, ATRA has recently been used to treat APL, achieving high complete remission rates. However, ATRA has limited success as a single agent in the treatment of other hematopoietic malignancies such as acute myeloid leukemia (AML). Combinations of adenosine or ATP and differentiation inducers ATRA, VD3 and PMA were evaluated for their abilities to induce the differentiation of leukemia THP-1 cells. ATRA combined with adenosine or ATP significantly increased the expression of CD11b in THP-1 cells compared with ATRA alone, suggesting that the combined treatment may have potential for the treatment of AML which is less sensitive to ATRA. In case of VD3 and PMA, in contrast, both VD3 combined with adenosine or ATP, and PMA combined with adenosine or ATP did not increase the expression of CD11b in THP-1 cells compared with VD3 alone or PMA alone, respectively.

PMA-induced differentiation is associated with alterations in cell adherence of THP-1 cells, although THP-1 cells are floating cells. In contrast, other differentiation inducers ATRA and VD3 did not affect cell adherence in THP-1 cells. Our analysis indicate that adenosine and ATP increased the expression of CD11b, without affecting cell adherence in THP-1 cells (data not shown). These data suggest that the mechanisms by which adenosine and ATP induce the differentiation may differ from the mechanisms by which PMA induces that. Leukemia cells are induced to differentiate into granulocyte in addition to monocyte/macrophage by a variety of inducers, losing the characteristics of leukemia cells and undergoing the suppression of multiplication.
Fig. 3 Effects of combinations of differentiation inducers and adenosine or ATP on differentiation in leukemia THP-1 cells. Cells were incubated with each differentiation inducer with adenosine or ATP for 72 h, followed by staining with anti-CD11b antibody. (a) Representative histograms of THP-1 cells treated with each differentiation inducer with adenosine or ATP as compared with each differentiation inducer alone-treated THP-1 cells are shown. Numbers indicate the Geo Mean fluorescence intensity value. The gray area shows the cells treated with each drug. (b) Geo Mean fluorescence intensity are shown with the mean±SE from three different specimens. *P<0.05, **P<0.01, when compared with adenosine or ATP-treated cells, respectively. #P<0.01, when compared with each differentiation inducer-treated cells, respectively.
Fig. 4 Effects of combinations of differentiation inducers and adenosine or ATP on cell number in leukemia THP-1 cells. Cells were incubated with each differentiation inducer with adenosine or ATP for 72 h. Cell number was determined by colorimetric MTS assay. The data from three different specimens are shown. **P<0.01, when compared with adenosine or ATP-treated cells, respectively. ## P<0.01, when compared with each differentiation inducer-treated cells, respectively.

Although further work will be needed to resolve the molecular mechanism of differentiation, it is important that adenosine and ATP induce differentiation in leukemia THP-1 cells regardless of cell types such as granulocyte, monocyte and macrophage.

On the other hand, the accumulation of extracellular adenosine in inflamed and damaged tissues and the immunosuppressive properties of cAMP-elevating adenosine indicate that signaling to A2a adenosine receptor (A2aR) on immune cells is a possible natural mechanism of inhibition and/or termination inflammation. Thus, adenosine is a critical part of the physiological negative feedback mechanism for limitation and termination of both tissue-specific and systemic inflammatory responses. In this study, we found that adenosine and ATP can induce the differentiation in leukemia THP-1 cells. In contrast, peroxisome proliferators-activated receptor γ (PPARγ) ligands have been proposed as possible therapeutics for inflammatory disease. However, PPARγ ligand failed to induce the differentiation as assessed by evaluating the expression of CD11b in leukemia THP-1 cells and U937 cells, respectively (our unpublished data). In this regard, it is likely that the abilities of anti-inflammatory responses might be independent of the abilities of differentiation induction. It thus appears that adenosine and ATP may induce the differentiation in human leukemia THP-1 cells, and may further increase the ATRA-induced differentiation in THP-1 cells. At this end, we believe that induction of differentiation by adenosine and ATP could have therapeutic applications in leukemia.

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References


アデノシンおよび ATP による白血病細胞 THP-1 の分化誘導

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アデノシンおよび ATP は炎症反応の制御に関与し、細胞発育、細胞分化およびアポトーシスにも影響を与える。本研究において、アデノシンおよび ATP によるヒト白血病細胞 THP-1 分化能について検討を行った。アデノシン 10^{-3} M において、CD11b の発現測定により、著明な THP-1 分化誘導が認められた。同様に ATP 10^{-3} M において、有意な THP-1 分化誘導が認められた。また、アデノシン 10^{-3} M および ATP 10^{-3} M において THP-1 細胞数の有意な抑制が認められた。次にわれわれは、all-traneretinoic acid (ATRA), 1,25-dihydroxy vitamin D3 (VD₃), および phorbol 12-myristate 13-acetate (PMA) といったような分化誘導剤によって分化させた THP-1 に対するアデノシンおよび ATP の影響について検討を行った。すると興味深いことに、アデノシン 10^{-3} M において ATRA 誘導性 CD11b の発現が有意に増強され、さらに ATRA はアデノシン誘導性 CD11b の発現を有意に増強した。同様に ATP 10^{-3} M においても ATRA 誘導性 CD11b の発現が促進された。これと比較して、VD₃ と PMA については、それぞれアデノシンおよび ATP との併用により CD11b のさらなる発現増強が認められた。以上の結果より、アデノシンおよび ATP はヒト白血病細胞 THP-1 の分化を誘導し、ATRA によりさらに分化誘導が増強されるということが示唆された。

キーワード：アデノシン、ATP、LPS、分化、CD11b