Lysis of Human Monocytic Leukemia Cells by Extracellular Adenosine Triphosphate: Mechanism and Characterization of the Adenosine Triphosphate Receptor

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The present study shows that extracellular adenosine triphosphate (ATP) has the capacity to mediate dose-dependent lysis of the monocytic leukemia cell line THP-1. The lysis, assessed by $^{51}$Cr release, was found to be selective for ATP, because adenosine diphosphate (ADP) or other nucleotides were less effective in their ability to lyse the cells. The amount of $^{51}$Cr released was particularly enhanced by the stimulation of the cells with 1,000 U/mL of interferon gamma (IFN-γ) for 3 days, and the sensitivity was time and dose dependent. Analysis of the mechanism of lysis indicated that the fully ionized form, ATP$^-$, mediated the lysis, because the addition of cation chelators or the absence of the divalent cations, Ca$^{2+}$ and Mg$^{2+}$, in the culture medium of a 6-hour $^{51}$Cr release assay increased the percent specific lysis. Therefore, the ATP receptors on THP-1 cells were classified as P$_2$ purinoceptors. Moreover, it is shown here that the Ca$^{2+}$/calmodulin complex plays a role in the regulation of the lysis by extracellular ATP of THP-1 cells, because antagonists of this complex, such as trifluoperazine or KN-62, were found to inhibit the ATP-mediated cell lysis.

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THE PROFOUND EFFECT of adenosine triphosphate (ATP) on cell physiology has been described over a period of years in several laboratories. In addition to physiologic modulation, ATP has recently been found to be lytic for ATP, because adenosine diphosphate (ADP) or other nucleotides were less effective in their ability to lyse the cells. The amount of $^{51}$Cr released was particularly enhanced by the stimulation of the cells with 1,000 U/mL of interferon gamma (IFN-γ) for 3 days, and the sensitivity was time and dose dependent. Analysis of the mechanism of lysis indicated that the fully ionized form, ATP$^-$, mediated the lysis, because the addition of cation chelators or the absence of the divalent cations, Ca$^{2+}$ and Mg$^{2+}$, in the culture medium of a 6-hour $^{51}$Cr release assay increased the percent specific lysis. Therefore, the ATP receptors on THP-1 cells were classified as P$_2$ purinoceptors. Moreover, it is shown here that the Ca$^{2+}$/calmodulin complex plays a role in the regulation of the lysis by extracellular ATP of THP-1 cells, because antagonists of this complex, such as trifluoperazine or KN-62, were found to inhibit the ATP-mediated cell lysis.

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MATERIALS AND METHODS

Materials. ATP (tissue culture grade), adenosine diphosphate (ADP), uridine triphosphate (UTP), cytosine triphosphate (CTP), thymidine triphosphate (TTP), guanosine triphosphate (GTP), inosine triphosphate (ITP), 2'-3'-O-(4-benzoylbenzoyl)adenosine 5'-triphosphate (BeATP), ATP$^-$, trifluoperazine (TTP; in 70% ethanol), 4,4'-disothiocyanatostilbene-2,2'-disulfonic acid (DIDS; solubilized in 70% ethanol), and 5'-p-fluorosulfonylbenzoyl-adenosine (FSBA) were obtained from Sigma Chemical Co (St Louis, MO). N-(6-aminohexil)-5-chloro-1-naphthalenesulfonamide (W-7; solubilized in 95% ethanol) and KN-62 (dissolved in dimethyl sulfoxide [DMSO]) were obtained from Calbiochem (La Jolla, CA), and 2-methyl-thio-adenosine (2MeSATP) was from ICN Biochemicals (Cleveland, OH). Human recombinant IFN-γ was generously provided by Genentech Corp (South San Francisco, CA) and human recombinant GM-CSF was a generous gift from Immunex (Seattle, WA). All plasticware was obtained from COSTAR (Cumberland, MA).

Cell culture. THP-1 cells (American Type Culture Collection, Rockville, MD) were cultured in RPMI-1640 supplemented with 10% fetal calf serum (FCS) (HyClone Labs, Logan UT), 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 5 mmol/L HEPES buffer, and 5 × 10$^{-5}$ mmol/L 2-mercaptoethanol, and will subsequently be referred to as complete medium. The cell density was 1 × 10$^6$ cells/mL at the initiation of culture and was
incubated at 37°C in a 5% CO₂ atmosphere for 3 days unless noted. All media and reagents contained less than 0.1 ng/mL of endotoxin as determined by the Limulus lysate assay (MA Biologics, Walkersville, MD).

Measurement of ATP-mediated cytotoxicity. A 6-hour ⁵¹Cr-release assay was used to measure the effect of ATP on cytokine-treated THP-1 cells, and was performed essentially as previously described.³ THP-1 cells were labeled with sodium [⁵¹Cr]chromate (Amersham, Arlington Heights, IL) for 1 hour in 0.5 mL of medium, washed, and then added to serial dilutions of ATP in microtiter wells at 1 × 10⁶ cells/well in a final volume of 0.2 mL in each well. All determinations were done in triplicate, and the SEM of all assays was calculated and was typically 5% of the mean or less. Student’s t-tests were performed to identify significant differences between treatments.

RESULTS

Cytotoxic effect of extracellular ATP. THP-1 cells were tested for sensitivity to lysis by a wide range of concentrations of ATP in a 6-hour ⁵¹Cr release assay (Fig 1). In addition to cells cultured in medium alone, THP-1 cells were incubated in GM-CSF or IFN-γ for 3 days and were also tested for sensitivity to ATP-mediated lysis. The effect of ADP on cytokine-treated and untreated THP-1 cells was included as a control for specificity. As shown, a marked increase in the release of radioactivity was observed for the IFN-γ-treated cells in the presence of 1.25 mmol/L ATP (58.2% ± 4.0% specific lysis) as compared with the GM-CSF-treated cells (3.1% ± 0.9% specific lysis) or cells cultured in medium alone (8.2% ± 2.2% specific lysis). The reaction appeared selective for ATP, because neither GM-CSF-treated cells nor the medium control cells responded to ADP. Only IFN-γ-treated cells showed a slight sensitivity (10.4% ± 0.2% specific lysis) to ADP at 1.25 mmol/L, which was unlike human IFN-treated macrophages in which no susceptibility to ADP-mediated lysis was noted.³

Time course of IFN-γ-induced effects. The timing of the addition of IFN-γ to the cultures was found to be a factor in the increased sensitivity to ATP-mediated lysis. The lysis in a 6-hour ⁵¹Cr release assay, induced by 1.25 mmol/L ATP, of THP-1 cells cultured with 1,000 U/mL of IFN-γ or GM-CSF for the indicated length of time was assessed. IFN-γ was able progressively to increase the sensitivity of THP-1 cells to ATP-mediated lysis from the baseline level of 12% ± 2% specific lysis, to 26% ± 2% at day 2, to 36% ± 3% at day 3, and increasing to 55% ± 4% at day 4 of incubation. However, the parallel incubation of THP-1 cells with GM-CSF for 4 days did not increase the sensitivity of these cells to ATP, and even appeared to be slightly, but not significantly, decreased with 9% ± 1% specific lysis. For further experimentation, 3 days of incubation with cytokines was selected as the optimal incubation time, as the viability of the cells begins to decrease to approximately 80% by day 4, presumably because of the antiproliferative effect of IFN-γ.

Dose of IFN-γ required to induce sensitivity. The effect of different concentrations of IFN-γ on the percent lysis was studied to identify the optimal concentration of cytokine needed to induce ATP-mediated lysis. THP-1 cells were cultured in the presence of the indicated concentration of IFN-γ (Fig 2) for 3 days before being tested for ATP sensitivity in a 6-hour ⁵¹Cr-release assay. As shown, increasing concentrations of IFN-γ induced increasing susceptibility of THP-1 to lower concentrations of ATP; ie, treatment of cells with 100 U/mL of IFN-γ resulted in 11% ± 2% specific lysis,

![Graph](image-url)

Fig 1. ATP/ADP dose-response for THP-1 cells. THP-1 cells were incubated for 3 days in medium alone, with 1,000 U/mL of IFN-γ or with 1,000 U/mL of GM-CSF before being used as target cells in a 6-hour ⁵¹Cr release assay. Numbers are means of triplicate determinations and the data are representative of three experiments that were performed with similar results. (△), medium; (ACCEPT), 1 U/mL; (●), 10 U/mL; (◆), 100 U/mL; (□), 1,000 U/mL.

![Graph](image-url)

Fig 2. Effect of IFN-γ concentration on the sensitivity of THP-1 cells to ATP. THP-1 cells were incubated in medium alone or with the indicated concentration of IFN-γ for 3 days before being used as target cells in a 6-hour ⁵¹Cr release assay. Numbers are means of triplicate determinations and the data are representative of three experiments that were performed with similar results. (△), medium; (ACCEPT), 1 U/mL; (●), 10 U/mL; (◆), 100 U/mL; (□), 1,000 U/mL.

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whereas 1,000 U/mL induced 42% ± 2% specific lysis at the same ATP concentration of 1.25 mmol/L. Therefore, for our subsequent experiments, the dose of IFN-γ used was 1,000 U/mL.

In studies using human macrophages, significantly less IFN-γ was required to induce ATP sensitivity (maximal activation was found using 100 U/mL as compared with 1,000 U/mL for THP-1 cells). To show that the response of THP-1 was specific for IFN-γ, THP-1 cells were stimulated with (1) medium alone; (2) 1,000 U/mL IFN-γ; (3) 1,000 U/mL of IFN-γ that had been neutralized using excess anti–IFN-γ monoclonal antibodies (MoAbs) (Genzyme Corp, Cambridge, MA) at 37°C for 1 hour before its addition to THP-1; and (4) anti–IFN-γ alone. Cultures were then incubated for 3 days and used as target cells in a 2-hour 51Cr release assay. The specific lysis of each of these cultures in the presence of 1.25 mmol/L ATP was 6% ± 1%, 45% ± 3%, 8% ± 1%, and 8% ± 1%, respectively, indicating that sensitization of THP-1 tumor cells by IFN-γ was specific for this cytokine. Finally, incubation of THP-1 cells with up to 1,000 U/mL of GM-CSF for 3 days did not differ significantly from a control culture incubated in the presence of medium alone (data not shown). Thus, the remaining experiments will be performed by comparing IFN-γ-treated cells with GM-CSF–treated THP-1 cells to compare the effects of different cytokines on these monocytic cells.

Time course of 51Cr release from ATP-stimulated THP-1 cells. We next investigated the early events that occurred after the exposure of THP-1 cells to extracellular ATP. The supernatants were collected at the indicated time points from IFN-γ–treated or GM-CSF–treated cells after stimulation with 1.25 mmol/L ATP (Fig 3). GM-CSF–treated THP-1 showed little 51Cr release after 2 hours of incubation with ATP and required 4 hours to show significant lysis, which steadily climbed to 26% by 24 hours. Further incubation up to 30 hours did not increase the maximal lysis of these cells by ATP (data not shown). IFN-γ–treated THP-1 cells, however, rapidly released the radioactivity into the supernatant fluids, which was maximal after the first 2 hours. The percent of 51Cr released after 24 hours was not significantly different from the percent released at 2 hours. Thus, there was a major difference between IFN-γ–treated and GM-CSF–treated THP-1 cells in the rapidity of the radiolabel release. Moreover, this difference in the sensitivity to ATP is maintained over time, as a longer incubation does not result in a more complete killing of GM-CSF–treated cells, which showed 26% specific lysis after 24 hours as compared with 72% in IFN-γ–treated cells incubated with ATP for the same length of time.

Effect of other nucleotides on THP-1 cells. To determine the specificity of the ATP stimulation, various nucleotides at a 2.5 mmol/L concentration were assessed for their efficacy against IFN-γ–treated THP-1 cells in a 5-hour 51Cr release assay (Table I). Of all the nucleotides tested, as well as adenosine, only ATP caused lysis greater than 10%, indicating that the ligand selectivity is the same as that which causes the lysis of normal human macrophages, as described previously. ATPγS, a poorly hydrolyzable ATP analogue, was half as effective as ATP, causing 29% specific lysis as compared with 57% lysis by ATP. These results indicate that the hydrolysis of ATP is not indispensable for the lysis of THP-1 cells.

Effect of divalent cations on ATP-mediated lysis of THP-1 cells. A growing body of data suggests the existence of specific cell surface receptors for ATP, and classification of these purinoceptors has been based on selective binding of agonists and antagonists of ATP. The presence of P2X purinoceptor has been described in rat mast cells, in mouse macrophages, and in transformed cell lines, and has been shown to mediate the permeabilizing effect of ATP on these cells. The observation that only ATP, but not aden-
osine, ADP, UTP, ITP, CTP, or GTP, can induce the lysis of THP-1 cells could suggest that the receptor for ATP on THP-1 cells belongs to the P2X class. Moreover, it has been shown that the ligand that activates the P2X receptor is the tetrabasic ion, ATP$^{4-}$, which is present as a minor equilibrium component in solutions containing divalent cations, to which ATP is normally complexed. Because of the high affinity of ATP$^{4-}$ for Mg$^{2+}$ and Ca$^{2+}$, the amount of ATP$^{4-}$ required to induce lysis depends on the divalent cation concentration in the medium. To determine the influence of divalent cations in ATP-mediated lysis of THP-1 cells, we measured the percent lysis of these cells by 2.5 mmol/L ATP in various concentrations of Ca$^{2+}$ and Mg$^{2+}$. The cytotoxicity assay was performed in a buffered salt solution comprising 135 mmol/L NaCl, 5 mmol/L KCl, 30 mmol/L HEPES, pH 7.4, containing 0.2% bovine serum albumin (BSA) and 2-mercaptoethanol, and lacking Ca$^{2+}$ and Mg$^{2+}$. Increasing amounts of cations were then added to the medium in the form of CaCl$_2$ and MgCl$_2$. As shown in Table 2, increasing the concentration of Ca$^{2+}$ and Mg$^{2+}$ from 1 to 4 mmol/L caused decreased release of $^{51}$Cr in the supernatant. Thus, as the effective concentration of ATP$^{4-}$ was decreased, the percent lysis of THP-1 was concomitantly lowered. Conversely, in the absence of micromolar concentrations of the cations, the percent specific lysis increased along with the higher concentration of the ATP$^{4-}$-ionic species. The same effect was observed with THP-1 cells treated with GM-CSF, although the percent specific lysis for the GM-CSF–treated cells (Table 2), in the presence of micromolar concentrations of cations, was lower than that of IFN–γ–treated cells.

Next, we evaluated the effect of chelating agents on ATP-mediated lysis of THP-1. The lytic effect of ATP was increased by the addition in the incubation medium of EDTA from 1 to 4 mmol/L, which chelated the extracellular free divalent ions and subsequently increased the relative concentration of ATP$^{4-}$. The addition of EDTA increased the lysis of THP-1, an effect that was most prominent at lower concentration of ATP. As little as 1 mmol/L of EDTA increased the lysis of THP-1 cells from 6.3% ± 0.8% to 19.1% ± 1.7%, with 0.31 mmol/L ATP. Increasing the concentration of EDTA to 4 mmol/L augmented the sensitivity to this concentration of ATP to 68.7% ± 1.7%, confirming that the active form of ATP was the tetrabasic ionic species, and defines the permeabilizing ATP receptor on THP-1 cells as the P2X purinoceptor.

**ATP-degrading activity on the THP-1 cell surface.** Filippini et al. have shown the existence of ATPases on the cell surface of cytolytic T lymphocytes. The difference in percent lysis of IFN–γ–treated, or GM-CSF–treated cells after exposure to ATP could be caused by a difference in activity of the ecto-ATPases on the surface of these cells, and it was possible that GM-CSF–treated THP-1 cells were more resistant to ATP because of elevated levels of surface ecto-ATPase activity. Because FSBA has been found to be a very efficient inhibitor of ecto-ATPases in the lymphocytes, THP-1 cells were pretreated for 30 minutes at 37°C with 10, 100, and 1,000 μmol/L FSBA before incubating for 4 hours in the presence of 2.5 mmol/L ATP. No difference in the percent $^{51}$Cr release was seen between the untreated THP-1 cells and the cells pretreated with FSBA. In addition, FSBA had no effect on the ATP-mediated lysis of IFN–γ–treated THP-1 cells. These results suggest that differences in ecto-ATPase activity was not the cause of the difference between IFN–γ–treated cells and GM-CSF–treated cells.

Effect of DIDS, a purinoceptor antagonist, on ATP-mediated lysis of THP-1. DIDS is an irreversible cross-linking reagent that inhibits anion exchange, and it has been shown to block the effects of ATP on all the physiologic responses by preventing the binding of ATP to a site on the plasma membrane. In particular, DIDS can selectively block the purinergic receptor for the ATP$^{4-}$ form. In view of the significance of these findings, we investigated the effect of DIDS on ATP binding in our system. GM-CSF–treated and IFN–γ–treated THP-1 cells were incubated for 15 minutes at 37°C with 100, 200, and 300 μmol/L DIDS before being washed and incubated for 4 more hours in the presence of 1.25 mmol/L ATP. The lysis of both types of cells was significantly blocked in a dose-dependent manner by DIDS (Fig 4). The lysis of IFN–γ–treated cells was 69% ± 2%, 41% ± 2%, 23% ± 1%, and 19% ± 1% in the presence of 0, 100, 200, and 300 μmol/L DIDS, respectively. At higher concentrations of DIDS, direct toxicity of this compound on THP-1 cells was noted. Similar inhibition of ATP-mediated lysis by DIDS was seen using GM-CSF–treated THP-1 cells, although lysis by ATP was not as pronounced. These results suggest that ATP may act on a common DIDS-sensi-

### Table 2. Effect of Divalent Cations on ATP-Mediated Lysis of THP-1 Cells

<table>
<thead>
<tr>
<th>% Specific Lysis ± SEM in the Presence of</th>
<th>0 mmol/L Mg$^{2+}$</th>
<th>1 mmol/L Mg$^{2+}$</th>
<th>2 mmol/L Mg$^{2+}$</th>
<th>4 mmol/L Mg$^{2+}$</th>
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<tr>
<td>IFN–γ–treated THP-1 (mmol/L Ca$^{2+}$)</td>
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<tr>
<td>0</td>
<td>48 ± 2</td>
<td>28 ± 1</td>
<td>13 ± 1</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>1</td>
<td>38 ± 2</td>
<td>13 ± 1</td>
<td>10 ± 1</td>
<td>12 ± 1</td>
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<td>2</td>
<td>0 ± 1</td>
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<td>4</td>
<td>0 ± 1</td>
<td>1 ± 1</td>
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<tr>
<td>GM-CSF–treated THP-1 (mmol/L Ca$^{2+}$)</td>
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<tr>
<td>0</td>
<td>13 ± 1</td>
<td>6 ± 1</td>
<td>0 ± 1</td>
<td>1 ± 1</td>
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<tr>
<td>1</td>
<td>1 ± 1</td>
<td>3 ± 1</td>
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<td>2</td>
<td>0 ± 1</td>
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<td>4</td>
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* THP-1 cells were incubated for 3 days in the presence of 1,000 U/mL of IFN–γ or GM-CSF before being used as target cells in a 6-hour Cr release assay using 1.25 mmol/L ATP as the lytic agent. The indicated concentrations of CaCl$_2$ and MgCl$_2$ were included in the assay medium (a buffered salts solution lacking Ca$^{2+}$ and Mg$^{2+}$). Data are representative of three experiments performed with similar results.
Fig 4. Dose-response relationship characterizing the inhibitory action of DIDS on ATP-induced lysis of THP-1 cells. THP-1 cells were cultured for 3 days with the indicated cytokines and used as a target cells in a 6-hour $^{51}$Cr release assay. Before adding 1.25 mmol/L ATP, the cells were incubated with the indicated concentration of DIDS for 15 minutes. Numbers are means ± SEM of triplicate determinations and data are representative of three experiments that were performed with similar results. (○), GM-CSF; (□), IFN-γ.

Fig 5. Effect of ATP analogues on lysis of THP-1 cells. THP-1 cells were cultured with IFN-γ for 3 days before being used as target cells in the presence of the indicated concentration of ATP or analogues. The mean ± SEM of triplicate determination is shown. Data are representative of three experiments that were performed with similar results. (○), ATP; (□), 2MeSATP; (♦), BzATP.

Ca$^{2+}$/calmodulin in the signal transduction pathway leading to the cell death of human macrophages (manuscript in preparation). Based on these data, we examined the role of Ca$^{2+}$/calmodulin in the cellular processes of THP-1 cells after exposure to ATP. Therefore, inhibitors of Ca$^{2+}$/calmodulin–linked enzymes were assessed for their ability to block ATP-mediated lysis. There are several mechanisms by which drugs might act to inhibit the action of Ca$^{2+}$/calmodulin. For example, W7 and TFP act by binding to the complex and modifying its activity.25 Figure 6 shows that

Fig 6. Effect of TFP on ATP-mediated lysis of THP-1 cells. Cytokine-treated THP-1 cells were incubated 15 minutes with the indicated concentration of TFP before being used as target cells in a $^{51}$Cr release assay with 1.25 mmol/L ATP. Numbers are means ± SEM of triplicate determinations and data are representative of three experiments that were performed with similar results. (○), GM-CSF; (□), IFN-γ.
TFP greatly inhibited the ATP-mediated lysis of THP-1 cells treated with IFN-γ or GM-CSF. For these assays, we used a concentration of 1.25 mmol/L ATP, which caused a specific lysis of 72% ± 3% for IFN-γ-treated cells and 24% ± 2% for GM-CSF-treated cells. Different concentrations of TFP were added for 15 minutes at 37°C to the cells before adding ATP for 5 more hours. TFP appeared to be more effective in inhibiting the ATP-mediated lysis of GM-CSF-treated THP-1 cells than IFN-γ-treated cells, because 2.5 μmol/L TFP suppressed lysis of the former cells by 64%, whereas 5.0 μmol/L concentration was required to elicit the same level of inhibition in the latter cells. Surprisingly, the other calmodulin antagonist, W7, was unable to block lysis induced by ATP of both GM-CSF–treated and IFN-γ–treated THP-1 cells (data not shown). Because the calmodulin antagonists TFP and W7 have been shown to interact with multiple enzyme systems, a third, more selective compound, KN-62, was also used in these assays. Although W7 and TFP inhibit most calmodulin-dependent enzyme activities by interacting with the Ca²⁺/calmodulin complex, KN-62 has been reported to be a selective inhibitor of Ca²⁺/calmodulin kinase II enzyme, because it binds directly to this enzyme.26 We found that KN-62 was able completely to block lysis of both IFN-γ–treated and GM-CSF–treated THP-1 cells (Fig 7). Serial dilutions of KN-62 were added to the cells for 15 minutes at 37°C before adding 1.25 mmol/L ATP and incubating for 6 hours. As little as 0.3 μmol/L of KN-62 was able significantly to inhibit the lysis of both GM-CSF–treated and IFN-γ–treated cells, with almost complete inhibition being noted at 2.5 μmol/L. It should be noted that there was no direct toxicity of these inhibitors against THP-1 cells at the concentrations used in these experiments. Additionally, the effect of TFP cells and KN-62 on the ATP-mediated lysis of THP-1 cells that were not stimulated with cytokines was not significantly different from the effect of these inhibitors on GM-CSF-stimulated cells (data not shown). Thus, these findings suggest that there is an involvement of Ca²⁺/calmodulin in the ATP-mediated lysis of THP-1 cells whether or not these cells were activated with cytokines.

**DISCUSSION**

The present investigation was designed to characterize the mechanism of ATP-mediated lysis of the monocytic cell line THP-1. The lysis induced by ATP has been described for various cell types, although the mechanism were not characterized. The experiments reported here showed that ATP has potent cytotoxic effect on THP-1 tumor cells. This result appeared to be selective for ATP, because adenosine, ADP, UTP, CTP, GTP, and ITP produced no lytic effects. For IFN-γ–treated cells, the ATP-mediated lysis was proportional to the concentration of this purine nucleotide in the medium and was maximal when the concentration of ATP was 2.5 mmol/L or more. IFN-γ, in fact, greatly increased the sensitivity of the cells to ATP.

The sensitization of THP-1 cells in the presence of IFN-γ was time and concentration dependent, as the maximal sensitivity to ATP was reached after 3 days with 1,000 U/mL IFN-γ. Not only the sensitivity to ATP was increased, but also the kinetics of the lysis was faster, as more than 70% of the 51Cr was released from IFN-γ–treated THP-1 cells within 2 hours after the addition of ATP. For GM-CSF–treated cells, the release of 51Cr in the supernatant was gradual and the maximal lysis after 5 hours was under 30%.

These differences could be explained by a relative decrease in the expression of ecto-ATPase activity on the cell surface after treatment with IFN-γ or by a differential expression of ATP receptors either in the number or in their affinity for ATP. From our data, it seemed to be unlikely that the ecto-ATPases can degrade the lytic agent, as the addition of FSBA, which is known to inhibit the ecto-ATPase activity of cytotoxic T lymphocyte (CTL),27 to the culture medium of GM-CSF–stimulated THP-1 cells did not increase the percent of lysis, ie, 18% ± 1% in the presence of 2.5 mmol/L ATP, to the level observed for IFN-γ–treated THP-1 cells, which was 72% ± 2% at the same concentration of ATP. Thus, it seems more likely that a surface receptor for ATP was involved in the increased sensitivity of IFN-γ–treated THP-1 cells.

The presence of specific surface ATP receptors has been described by numerous reports in the literature in the past several years. As summarized in a review by Dubyak,27 there are at least three classes of ATP-specific receptors grouped on the basis of their selectivity for various nucleotides and their interaction with agonists or antagonists. P₂X is the class of receptors responsible for the formation of nonselective pores permeable to ions and metabolites up to 1 Kd in mass and leading to cell death, and it is reported to be the ligand that mediates the lysis of J774 mouse macrophages28 and of EL4 thymoma and P815 mastocytoma cell lines.15 This receptor has been shown to bind the tetrabasic anion ATP₄⁻ form.

From our studies, the same anionic form seems to be the
ligand of the ATP receptor of THP-1 cells, which leads to cell lysis. By modulating the concentration of the ATP<sup>4+</sup>-form in the culture medium with chelating agents or with Ca<sup>2+</sup>-free and Mg<sup>2+</sup>-free medium, the lysis of THP-1 cells can be regulated. The observation that the lysis was modulated equally in both GM-CSF-treated or IFN-γ-treated cells supports the suggestion that the ATP<sup>4+</sup>-form was recognized by the same or similar receptors in both cell types. Moreover, the suppressive effect of DIDS on THP-1 cell lysis by ATP and the ability of DIDS to block P<sub>Zz</sub>-type purinoceptors in rat parotid acinar cells<sup>19</sup> seem to confirm that the anionic form of ATP is the active receptor ligand for the purinoceptor on THP-1 cells.

The effect of ATP analogues, such as 2MeSATP, which binds the P<sub>z</sub> receptor with high affinity,<sup>11</sup> and BzATP, which is recognized by both P<sub>z</sub> and P<sub>zV</sub> receptors,<sup>25</sup> provide support that P<sub>z</sub> purinoceptors are the receptors involved in the ATP-mediated lysis of THP-1 cells. Their insensitivity to 2MeSATP compared with their high levels of lysis caused by the analogue BzATP provide evidence for the central role of P<sub>z</sub>-type receptors in our system.

The molecular pathway that mediates ATP-induced cell death is unknown. We investigated the role of calmodulin in the signal transduction mechanism leading to cell death by using the metabolic inhibitors TFP and W7, which have been widely used to study Ca<sup>2+</sup>/calmodulin-dependent enzyme systems.<sup>23,29</sup> The activity of the calmodulin antagonist TFP to block the lysis of THP-1 cells suggests the involvement of calmodulin protein in the ATP-mediated lysis, although the inefficacy of W7 brings to our attention the possibility that these inhibitors could interact with calmodulin via more than one mechanism. Moreover, it has been shown that calmodulin inhibitors, such as W7 and TFP, have different selectivity for the hydrophobic sites on calmodulin and might therefore interact differently with this calcium-binding protein.<sup>30,31</sup>

The efficacy of the more selective inhibitor KN-62, which binds directly to the enzyme Ca<sup>2+</sup>/calmodulin kinase II and not to calmodulin alone,<sup>26</sup> in blocking THP-1 cell lysis provides further evidence that calmodulin is involved in the ATP-mediated lysis of both IFN-γ- and GM-CSF-treated cells. Obviously, the involvement of this enzyme in the pathway leading to cell death warrants further investigation.

In conclusion, there were many similarities between the lytic effect of ATP on human macrophages<sup>5</sup> and the monocytic cell line THP-1. Such findings prompt us to speculate that, in our model, the physiologic role of ATP may be the downregulation of macrophages that have been stimulated with IFN-γ during an immune response. It would be interesting to determine the relative expression of the P<sub>Zz</sub> purinoceptor from the late myeloblast stage through the terminally differentiated stages represented by circulating monocytes and how stimulation with IFN-γ might modulate this receptor. From the data presented in this article, it is tempting to speculate that IFN-γ treatment of THP-1 cells increases the expression of the putative ATP receptor. This is supported by the observation that little difference, other than increased sensitivity to ATP lysis, was noted between IFN-treated and IFN-untreated cells; ie, all cells responded similarly to the range of inhibitors used in this study. However, until the P<sub>Zz</sub> purinoceptor can be quantitated, the relationship between IFN-γ and the ATP receptor on myeloid cells will remain unidentified.

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Lysis of human monocytic leukemia cells by extracellular adenosine triphosphate: mechanism and characterization of the adenosine triphosphate receptor

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