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Purine Metabolites Suppress Proliferation of Human NK Cells Through a Lineage-Specific Purine Receptor

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NK cell proliferation is suppressed in some patients with cancer by unknown mechanisms. Because purine metabolites released into the extracellular space during cell lysis may affect cell function, we hypothesized that these metabolites could serve as feedback regulators of NK cell proliferation. Sorted NK (CD56\(^+\)/CD3\(^-\)) cells were incubated with IL-2 (1000 U/mL) in a 4-day thymidine uptake assay with or without 10–10,000 \(\mu\)M of nucleotides. Adenine nucleotides inhibited NK cell proliferation, with ATP = ADP > S'-adenylylimidodiphosphate > AMP = adenosine; ADP-ribose and nicotinamide adenine dinucleotide, but not nicotinamide or UTP, caused a dose-dependent suppression of thymidine uptake. A total of 100 \(\mu\)M ATP, a concentration that induced a maximal (80%) inhibition of thymidine uptake, did not inhibit cytotoxic activity against K562 targets. Because NK cells retained the ability to lyse K562 targets 4 days after exposure to 500 \(\mu\)M ATP or 1000 \(\mu\)M adenosine, inhibition of thymidine uptake was not due to cell death. Incubation of NK cells with dibutyryl cAMP and forskolin also suppressed thymidine uptake. Cholera toxin and pertussis toxin suppressed NK cell proliferation. Pertussis toxin did not block the adenine nucleotide effects. Further, ATP, but not adenosine or other nucleotides, markedly increased intracellular cAMP in a dose-dependent manner. The ATP-induced increase in cAMP was specific to cytolytic cells, because CD19\(^+\) B cells and CD4\(^+\) T cells did not increase their intracellular cAMP. These studies demonstrate that NK proliferation is regulated through purine receptors by adenine nucleotides, which may play a role in decreased NK cell activity. The response to adenine nucleotides is lineage-specific.

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Human NK cells comprise a small population of PBLs, phenotypically identified as CD56\(^+\)/CD3\(^-\) cells, that possess the unique capacity to kill virus-infected or transformed cells in an HLA-unrestricted manner (1–3). A role for NK cells in tumor surveillance has been postulated based on the finding of NK cell defects in patients with solid tumors and leukemia (4, 5). In chronic myelogenous leukemia, we have shown progressive functional defects in NK proliferation, cloning frequency, and cytolytic function as the disease progresses from chronic phase to more advanced stages (6). In most patients, these defects are reversible and can be corrected in vitro by IL-2, raising the possibility that IL-2-based immunotherapy may play a role in cancer treatment (7). In fact, several human clinical trials have evaluated the effects of IL-2 infusion, with or without ex vivo activated lymphocytes, on tumor progression (8–11). Effector cell dysfunction in patients with cancer is not unique to chronic myelogenous leukemia; many types of cancers have been shown to impair immune function, which further complicates cancer treatments.

In addition to IL-2, other factors that enhance NK cell function have been described (12), although an increasing number of factors that inhibit NK function appear to be equally important in NK physiology and possibly in effective immunotherapy. For example, receptors on NK cells that recognize components of class I MHC alleles inhibit subsequent target lysis (13). In addition, we have shown that soluble factors released from target cells as well as direct contact with accessory cells resulted in a mixed effect on NK cell function (14). Accessory cells enhanced long-term (4 wk) proliferation but, paradoxically, resulted in an early (4 day) inhibition of proliferation. Part of the early inhibitory effect was due to activation of latent TGF-\(\beta\) by extracellular matrix thrombospondin, but other TGF-\(\beta\)-independent mechanisms were also involved.

NK cell-induced lysis of cells, perhaps at sites of tumor destruction or tissue inflammation, releases intracellular contents into the extracellular space. Purine metabolites released from intracellular stores appear to be involved in the regulation of immune function. In fact, treatment of mouse CTLs with nicotinamide adenine dinucleotide (NAD)\(^3\) (10 mM), but not ADP-ribose, AMP, or adenosine, resulted in the ADP-ribosylation of membrane proteins and in an inhibition of CTL proliferation (15). Only a partial inhibition of cytotoxicity was observed following incubation with higher concentrations of NAD (100 mM). It was shown subsequently that the inhibition of CTL proliferation was associated with a decrease in intracellular tyrosine phosphorylation mediated by p56\(^{Lck}\) (16). In addition, extracellular ATP affects signaling in lymphocytes by acting on P2 purinergic receptors, which opens membrane ion channels or activates G protein-coupled phospholipases (17). In fact, ATP and P2 receptors appear to be involved in triggering apoptosis during clonal deletion of thymocyte subsets in the thymus. Extracellular adenosine, however, induces an increase in intracellular Ca\(^2+\) or cAMP by signaling through P1 purinoceptors.

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In this paper, we investigate the effects of exogenous purine metabolites on the function of purified human NK cells and attempt to determine their mechanism of action.

Materials and Methods

Materials

NAD, ADP-ribose, nicotinamide, UTP, ATP, ADP, 5′-adenylylimidodiphosphate (AMP-PNP), AMP, adenosine, dibutyryl cAMP, forskolin and isobutylmethylxanthine were obtained from Sigma (St. Louis, MO); cholera toxin (CT), cholera toxin B subunit (CTB), pertussis toxin (PT), and PT B subunit (PTB) were provided by List Biological Laboratories (Campbell, CA), 7-aminomethylxymycin D (7-AAD) was purchased from Calbiochem (La Jolla, CA). K562 and Raji cells were obtained from the American Type Culture Collection (Manassas, VA); [3H]Thymidine and sodium chromate-51Cr were provided by DuPont (Wilmington, DE). rIL-2 was a generous gift from Amgen (San Diego, CA).

Study population

Peripheral blood from normal donors was obtained following the guidelines approved by the Committee on the Use of Human Subjects in Research at the University of Minnesota. PBMCs were obtained by Ficoll-Hypaque (specific gravity 1.077) (Sigma) density gradient centrifugation.

Purification of lymphocytes

NK cells were isolated from PBMCs using the MACS selection system (Miltenyi Biotec, Auburn, CA) as described by the manufacturer. Enriched cells were labeled with FITC-conjugated anti-CD3 and PE-conjugated anti-CD56 mAbs (250 ng/106 cells) (Becton Dickinson, San Jose, CA) and sorted on a FACStar Plus flow cytometer (Becton Dickinson) equipped with a Consort 32 computer (Hewlett Packard, Rolling Meadows, IL) into a CD56+CD3− population. The CD56+CD3− subpopulations were defined using established criteria for CD56+CD3− cells (18, 19). FITC-CD19+ B cells, PE-CD8+ (Becton Dickinson) T cells, and FITC-CD4+ (Becton Dickinson) T cells were purified from PBMCs using flow cytometry.

[3H]Thymidine uptake assay

Resting or activated NK cells that had been expanded long-term by incubation with IL-2 (1000 U/ml) for 18–24 days were incubated in 96-well plates (0.5–1.0 × 104/well) in a 2:1 DMEM/Ham's F12-based NK cell medium supplemented with 10% heat-inactivated human AB serum (North American Biologicals, Miami, FL) as described previously (14). IL-2 (1000 U/ml), adenine nucleotides, and toxins were added at the time of incubation. All assays were done in triplicate, and the percentage of lysis was determined. Cytokine levels were determined using K562 cells in a 4-h 51Cr release assay. E:T ratios ranged in a dose-dependent manner (Fig. 2), whereas nicotinamide (1 mM; 151 ± 20% of control) were inactive at concentrations of ≤1 mM.

Viability, cytotoxicity, and cytokine assays

The viability of NK cells, incubated without or with adenine nucleotides, was determined by trypsin blue exclusion. Cell viability was further analyzed using 7-AAD as described previously (20). NK cell cytotoxicity was assayed using K562 cells in a 4-h 51Cr release assay. E:T ratios ranged from 4:1 to 0:8:1. Target cells were labeled with 200 μCi of sodium chromate-51Cr for 60–90 min. All assays were done in triplicate, and the percentage of lysis was determined. Cytokine levels were determined using ELISA kits for TNF-α and IFN-γ according to the manufacturer's recommendations (R&D Systems, Minneapolis, MN).

Determination of intracellular cAMP concentration

Sorted NK cells, Raji cells, or other purified lymphocyte populations were incubated at 37°C under 5% CO2 in 300 μl of NK cell medium (without IL-2) with or without adenine nucleotides or toxins for 1–180 min, washed in PBS, centrifuged, suspended in 50 μl of lysis buffer (PBS containing 1 mM EDTA, 1 mM DTT, 250 mM sucrose, 1× complete protease inhibitor (Boehringer Mannheim, Indianapolis, IN), and 0.25 mM sodium butyrylthymine), and frozen in liquid nitrogen. Samples were stored at −80°C until use. Intracellular cAMP content was determined using the cAMP [3H] assay system (Biotrak Assay, Amersham, Arlington Heights, IL). Lysates (106 cells) were thawed, and the entire sample was assayed according to the manufacturer’s recommendations. [3H]cAMP was quantified in a scintillation counter (LS6500, Beckman, Schaumburg, IL), and the cAMP concentration is reported as picomoles per sample based on a standard curve (0–16 pmol cAMP) established for each assay.

Statistics

Data from multiple experiments are reported as means ± SEM. Significance levels were determined by a two-sided Student's t test.

Results

Sorted CD56+/CD3+ NK cells were incubated with IL-2 (1000 U/ml) with or without nucleotides (10–1000 μM) in a 4-day thymidine uptake assay (14). Adenine nucleotides suppressed thymidine uptake, with ATP and ADP being the most inhibitory (Fig. 1); AMP-PNP, a nonhydrolyzable analogue of ATP, also inhibited NK cell proliferation. In the presence of 50 μM of nucleotide, thymidine uptake relative to that of control cells (equalling 100%) was ATP (52 ± 9%) = ADP (49 ± 9%) = NAD (57 ± 9%) > ADP-ribose (77 ± 7%) = AMP-PNP (76 ± 13%) > adenosine (103 ± 9%) = AMP (120 ± 20%), consistent with the notion that the nucleotides exert their effect through a P2 (ATP) purinergic receptor. NAD and ADP-ribose also suppressed thymidine uptake in a dose-dependent manner (Fig. 2), whereas nicotinamide (1 mM; 151 ± 20% of control) and UTP (1 mM; 146 ± 26% of control) were inactive at concentrations of ≤1 mM.

The cytolytic function of NK cells was tested by their ability to lyse K562 cells. Freshly isolated resting NK cells (without IL-2 activation) were incubated with target cells in a 4-h 51Cr release assay. ATP at concentrations of >0.5 μM inhibited lysis, a concentration that was 5-fold higher than the 0.5 μM required for 50% inhibition of thymidine uptake (Fig. 3A). When ATP was added to resting NK cells 16 h before the addition of K562 cell targets (n = 2, data not shown), inhibition was less than when ATP was added at the start of the 4-h cytotoxicity assay, suggesting that the effect of ATP on cytotoxicity was transient; degradation of ATP or ATP receptor desensitization may occur during a prolonged incubation.

Adenosine is toxic to lymphocytes (21), and the effect of adenine nucleotides on thymidine uptake does not distinguish between inhibition of proliferation or toxic events leading to cell death. In the 4-day thymidine uptake experiments, however, >90% of treated and control cells were viable by trypsin blue exclusion.
In other experiments, NK cells were stained with anti-CD56 Ab and 7-AAD, a dye that stains cells with varying degrees of membrane damage, following a 60-h incubation with 100 μM ATP or 500 μM adenosine. There was no difference in NK cell staining with 7-AAD among control and ATP- or adenosine-treated cells. In the entire population of CD56^+ cells, 5% stained with 7-AAD. Trypan blue exclusion and 7-AAD staining rely on membrane integrity as a measure of viability but do not exclude the toxic effects of the nucleotides that may severely impair cell function. Therefore, we assessed NK function following treatment with ATP or adenosine. Sorted NK cells incubated for 4 days with IL-2 (1000 U/ml) and 500 μM ATP or 1 mM adenosine (concentrations ten times the lowest concentration found to inhibit thymidine uptake) demonstrated no differences in cytolytic activity against K562 cells on a per cell basis (Fig. 3B) despite significant inhibition of thymidine uptake, which was 26 ± 5% and 8 ± 0.7% of control, respectively.

Inhibition of proliferation may be influenced by the presence of NK cell subsets that are known to differ functionally. For example, CD56^bright cells, a subset possessing unique functional characteristics, have been postulated to represent NK cells that are more immature than the CD56^dim population (22). Further, activated NK cells, such as those generated following exposure to IL-2, may have a different proliferative response to ATP than do resting NK. We purified CD56^dim and CD56^bright cells and generated IL-2-activated NK (ANK) cells to test the effect of ATP on proliferation. ATP inhibited thymidine uptake by freshly sorted CD56^dim and CD56^bright cells in a dose-dependent manner, similar to that shown in Fig. 1 for the total CD56^+ population. ATP inhibited CD56^bright cells more than CD56^dim cells; it had a smaller effect on activated NK cells (Fig. 4).

In addition to cytotoxicity and proliferation, NK cells function to modulate immune responses by the production of cytokines. The TNF and IFN-γ produced by IL-2-activated NK cells were measured in the medium after 72 h of stimulation with or without the same concentration of nucleotides that resulted in an inhibition of proliferation. As expected, compared with samples of resting NK cells, IL-2 activation significantly increased IFN-γ production from 0.4 to 625 pg/ml (n = 4) and TNF from 0.3 to 116 pg/ml (n = 4). Adenosine significantly inhibited IL-2-induced TNF production by 60 ± 15% (p = 0.03), whereas ATP inhibition of TNF generation was more variable among experiments (46 ± 21%, p > 0.05). Adenosine and ATP did not significantly influence IL-2-induced production of IFN-γ by NK cells. Although adenosine diminished TNF production in response to IL-2, the high concentration required to generate a detectable cytokine response by the

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**FIGURE 2.** NAD and ADP-ribose (ADP-R) inhibit thymidine uptake by NK cells. Freshly sorted NK cells (10^4) were plated with IL-2 (1000 U/ml) and the indicated concentration of nicotinamide, NAD, or ADP-R. After 4 days, cells were incubated with [^3]H]thymidine; uptake was determined after 16 h. Results are expressed as the percentage of control (IL-2-stimulated cells without nucleotide) thymidine uptake. Inhibition of thymidine uptake was significant with 50 μM NAD or ADP-R. Data are means ± SEM of triplicate values from five experiments.

**FIGURE 3.** A. Effect of ATP and adenosine on NK cell cytolytic function. Freshly sorted resting NK cells (without IL-2) were added to ^51^Cr-labeled K562 cells at an NK E:T K562 cell ratio of 4:1. Cells were incubated without additions or with the indicated concentrations of ATP or adenosine, and chromium release was determined after 4 h (n = 3). ATP significantly suppressed cytotoxicity at 500, 1000, and 2000 μM (p = 0.046, p = 0.035, and p = 0.05, respectively), whereas inhibition by adenosine was not statistically significant at any of the concentrations tested. B. NK cell cytolytic function tested 4 days after the addition of ATP and adenosine. Sorted NK cells (0.5 × 10^6 cells/ml) were incubated with IL-2 alone as a control, with IL-2 plus 500 μM ATP, or with IL-2 plus 1 mM adenosine. After 4 days, NK cells were counted and tested against K562 cells in ^51^Cr release assays. In parallel experiments, NK cells (10^4) from the same donors were used in thymidine uptake assays as indicated (n = 3).
methods used in these experiments may mask a more pronounced effect of adenosine on cytokine production.

Purine receptors signal through both cAMP-dependent and -independent mechanisms. Before addressing this question directly, we determined the effects of agents that increase intracellular cAMP on thymidine uptake in NK cells. A dose-dependent inhibition of thymidine uptake was observed in cells grown in the presence of dibutyryl cAMP or forskolin (Fig. 5A). Further, CT (1 μM), which activates the stimulatory guanine nucleotide-binding protein (Gαs) and increases adenylyl cyclase activity, but not CTB, inhibited thymidine uptake (Fig. 5B). Inhibition of thymidine uptake was significantly greater when CT was added to cells incubated with 100 or 200 μM ATP (p = 0.017 and p = 0.007, respectively) compared with the inhibition observed with ATP alone. Addition of ATP to CT-treated cells resulted in greater inhibition than that observed with CT alone (for 100 μM ATP, p = 0.031; for 200 μM ATP, p = 0.013). CTB had no effect on thymidine uptake. Treating NK cells with PT, which uncouples Gαi, Gαo, and Gαt from their receptors, did not abrogate the inhibitory effect of ATP. Although PT inhibited thymidine uptake (p < 0.001), no significant difference was observed when ATP-treated cells were incubated with or without PT (Fig. 5C), suggesting that ATP may be acting through a Gi-independent pathway. PTB inhibited NK cells (Fig. 5C), which is consistent with the notion that PTB is immunomodulatory (23).

Intracellular cAMP levels were determined using a competitive protein binding assay. In this assay, there is reportedly little interference from other naturally occurring nucleotides, although ATP may cross-react at concentrations of >5 mM. To confirm assay sensitivity, varying concentrations of ATP were exogenously added to samples before quantifying cAMP. In our assays, samples containing 0.25, 2.5, and 25 mM ATP yielded values of 0 ± 0, 0.1 ± 0.9, and 1.1 ± 0.34 pmol of cAMP, respectively (n = 5). Because cells were washed before the measurement of cAMP, the effects of ATP in this assay were not significant. In sorted NK cells (10⁵) incubated with adenine nucleotides or other compounds, 100 μM ATP, but not other nucleotides, increased cAMP levels after 20 min (Fig. 6A). Although forskolin increases cAMP by stimulating adenylyl cyclase, the increase observed in NK cells was variable and not statistically significant. NK cells, however, demonstrated a time- and dose-dependent increase in cAMP in response to 10–1000 μM extracellular ATP (Fig. 7). CT, but not CTB, likewise increased cAMP after 60 or 180 min (Fig. 6B). Raji (B lymphoblastoid) cells, conversely, exhibited a brisk increase in cAMP following treatment with forskolin and adenosine, but not ATP (Fig. 6C). These data are consistent with the idea that lymphocyte responses to nucleotides and compounds that increase cAMP may be lineage-specific.

NK cells, B cells, and T cells isolated from the same donor by flow cytometry were used to determine whether primary lymphocytes exhibit lineage-specific responses to nucleotides as suggested from data using Raji cells. Lymphocytes were incubated without additives or with 1000 μM adenosine, ATP, NAD, or ADP. As noted above, ATP significantly increased NK cell cAMP levels compared with controls or cells incubated with other metabolites (Table I). In contrast, CD19⁺ B cells and CD4⁺ T cells did not exhibit increased cAMP content in response to any of the metabolites tested. Like NK cells, CD8⁺ T cells demonstrated increased intracellular cAMP levels following incubation with ATP.

![FIGURE 4](https://www.jimmunol.org/)

**FIGURE 4.** Effects of ATP on [³H]thymidine uptake by CD56⁺bright, CD56⁺dim, and activated (IL-2-stimulated) NK cells. Thymidine uptake was measured in freshly sorted CD56⁺bright, CD56⁺dim, and activated NK cells that had been incubated with IL-2 (1000 U/ml) for 18 days (ANK) following treatment with the indicated concentrations of ATP (n = 3 separate experiments performed in triplicate). CD56⁺bright NK cells were inhibited more than CD56⁺dim NK cells by 100 μM ATP (p = 0.008); both were inhibited more than ANK and CD56⁺dim cells; p = 0.005 between ANK and CD56⁺bright cells.

![FIGURE 5](https://www.jimmunol.org/)

**FIGURE 5.** Effects of forskolin or dibutyryl cAMP on thymidine uptake. A, Freshly sorted NK cells were incubated with or without the indicated concentration of forskolin (○) or dibutyryl cAMP (●) before measurement of thymidine uptake 4 days later (n = 4). B, NK cells were incubated with the indicated concentration of ATP without toxin, plus 1 μg/ml CT, or plus 1 μg/ml CTB (n = 4). C, NK cells were incubated with the indicated concentration of ATP without toxin, plus 1 μg/ml PT, or plus 1 μg/ml PTB (n = 5). Data are means ± SEM of the average of triplicate wells from the indicated number of experiments. All additions were at the time of initial plating.
the magnitude of the response, however, was less than that of NK cells. The increase seen in 2 × 10^5 CD8^+ cells was smaller than that in 1 × 10^5 NK cells (Table I).

**Discussion**

Purine receptors have been identified on the surface of many cells (24-26), including lymphocytes (27), where they might play an important role in the regulation of immune function (28). Purine nucleotides may be released at sites of tissue damage or inflammation, where they could modulate the immune response. Incubating human NK cells with extracellular purine nucleotides resulted in a dose-dependent inhibition of thymidine uptake, which is a sensitive measure of IL-2-induced proliferation. The inhibition of thymidine uptake in our studies was not due merely to cell damage. NK cells incubated with IL-2 and high concentrations of ATP or adenosine maintained cytotoxic function that was comparable with that of control cells after correction for differences in cell number. This inhibition of thymidine incorporation induced by extracellular adenine nucleotides was in fact due to an inhibition of proliferation, because treated cells maintained their capacity to lyse tumor target cells. IL-2-induced proliferation was more profoundly affected by ATP and adenosine than was NK cell cytotoxicity, suggesting that these functions are not coupled in the cell responses to extracellular purine metabolites.

The findings that both NAD and ADP-ribose inhibited human NK proliferation are in contrast to data from Wang et al., which demonstrated that NAD, but not ADP-ribose, inhibited the proliferation of mouse CTLs (15). In their experiments, inhibition of thymidine uptake and, at higher NAD concentrations, inhibition of cytotoxicity, was associated with the failure of CTLs to form conjugates with targets. The immune regulation of NAD on murine CTLs was associated with the actions of a cell surface GPI-anchored NAD:arginine ADP-ribosyltransferase. In the current studies using human NK cells, inhibition of proliferation in the presence of ADP-ribose is consistent with the cellular effects mediated through purinergic receptors rather than ADP-ribosylation. The disparity between the two findings may be explained by species differences (human vs murine) or by differences in the lymphocyte populations studied (NK cells vs CTLs).

The purine nucleotides that have been reported to have multiple biological effects on lymphoid cells (29, 30) include ATP, which induced cell-mediated killing of some tumor targets (31). Ecto-ATPases protected effector cells from released ATP (32). In other studies, ATP, but not UTP, adenosine, AMP, ADP, or AMP-PNP, inhibited the cytotoxicity of resting NK cells in a short assay (1-2 h), whereas cells stimulated by IL-2 were more resistant to inhibition (32, 33). Inhibition of NK cell lysis was not a result of interference with target cell binding, but appeared to be due to postrecognition signaling events (34). In our experiments, minimal inhibition of K562 lysis with low ATP concentrations may have been the result of the longer (4 h) incubation during the cytotoxicity assay.

Purinergic receptors may signal by increasing intracellular cAMP. Incubating NK cells with CT, forskolin, or dibutyryl cAMP, agents known to increase cAMP (35), resulted in an inhibition of proliferation. Although PT has been shown to inhibit NK

**FIGURE 6.** Determination of intracellular cAMP in NK cells. A. Sorted NK cells (10^6) were incubated for 20 min without additions (control) or with 50 μM forskolin (Forsk), 100 μM ATP, 1 mM adenosine, 100 μM AMP-PNP, 100 μM NAD, or 200 μM ADP-ribose (ADPR), washed, suspended in lysis buffer, and frozen in liquid nitrogen before determination of cAMP levels. Data are means ± SEM for the indicated number of experiments. B, NK cells were incubated for the indicated time in CT (10 μg/ml) or CTB (10 μg/ml) before analysis as in A. C, Raji cells were incubated for 20 min with or without 50 μM forskolin (Forsk), 1 mM ATP, or 1 mM adenosine (ADO) before analyses as in A (mean of two experiments).

**FIGURE 7.** Effect of ATP on cAMP content of NK cells. After incubation for the indicated times with or without ATP, NK cells (1–1.5 × 10^5) were lysed and assayed for cAMP as described in Fig. 6. Data are means ± SEM; p values are calculated against control cells incubated without ATP (no ATP).
cell cytotoxicity (36), our data demonstrate that PT or PTB inhibited NK proliferation. The addition of PT to PTB-treated cells did not abrogate this affect, however, suggesting a Gi-independent mechanism of inhibition. Detection of increased intracellular cAMP content following incubation with ATP, but not adenosine or other metabolites, is consistent with the hypothesis that ATP acts through a P2 receptor. Interestingly, the increased levels of cAMP induced by ATP were most significant in NK cells, whereas there was less of a response in CD8\(^+\) T cells and no effect in CD4\(^+\) T cells or B cells. These data are compatible with the presence of a P2 receptor that is expressed specifically in cytolytic lymphocytes. Inhibition of proliferation by other nucleotides, in addition to ATP, may be mediated by multiple mechanisms, because nucleotides that were potent inhibitors of NK cell proliferation (e.g., NAD and AMP-PNP) did not measurably increase intracellular cAMP concentrations. Further, the fact that adenosine and, to a lesser extent, ATP inhibited IL-2-mediated TNF but not IFN-\(\gamma\) production demonstrated that adenosine nucleotides modulate specific cellular pathways and are not inducing a generalized inhibition of function.

Clinically, immune suppression, as measured by inhibition of IL-2-induced NK cell proliferation, is observed in patients with chronic myelogenous leukemia, presumably by interaction of NK cells with malignant cells or a soluble factor from these cells. Similar observations of immune suppression have been made in other cancers. Although determining local concentrations of extracellular purine metabolites in vivo has been difficult due to their other cancers. Although determining local concentrations of extracellular purine metabolites in vivo has been difficult due to their production demonstrated that adenosine nucleotides modulate specific cellular pathways and are not inducing a generalized inhibition of function.

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Understanding the effects of the interactions between malignant and immunoregulatory cells is essential for the successful treatment of cancers.

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